Spatiotemporal Pattern of Phenology across Geographic Gradients in Insects

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CONTENTS

SUMMARY

Phenology – the timing of recurrent biological events – influences nearly all aspects of ecology and evolution. Phenological shifts have been recorded in a wide range of animals and plants worldwide during the past few decades. Although the phenological responses differ between taxa, they may also vary geographically, especially along gradients such as latitude or elevation. Since changes in phenology have been shown to affect ecology, evolution, human health and the economy, understanding phenological shifts has become a priority.

Although phenological shifts have been associated with changes in temperature, there is still little comprehension of the phenology-temperature relationship, particularly the mechanisms influencing its strength and the extent to which it varies geographically. Such questions would ideally be addressed by combining controlled laboratory experiments on thermal response with long-term observational datasets and historical temperature records. Here, I used odonates (dragonflies and damselflies) and Sepsid scavenger flies to unravel how temperature affects development and phenology at different latitudes and elevations.

The main purpose of this thesis is to provide essential knowledge on the factors driving the spatiotemporal phenological dynamics by (1) investigating how phenology changed in time and space across latitude and elevation in northcentral Europe during the past three decades, (2) assessing potential temporal changes in thermal sensitivity of phenology and (3) describing the geographic pattern and usefulness of thermal performance curves in predicting natural responses. Additionally, this thesis presents (4) a new behavior in odonates and (5) a commemoration of the 100th anniversary of a noteworthy book in odonatology.

In **Chapter I**, the phenological shift of adult odonates across latitude and elevation was examined with long-term observation data of 54 species in six European countries, historical temperature records and experimental studies on species-specific thermal performance curves. Geographic variation in phenological shifts is expected since the magnitude of recent climate warming varies across both space (latitude and elevation) and time (seasons). However, little information exists on whether the average temperature, fluctuation of temperature or physiological response to such temperature fluctuations is the best predictor of phenology. First, phenology has shifted across both latitude and elevation, but to a different extent (magnitude), increasing with

latitude and decreasing with elevation. Although average temperature explained some of the variation in spatiotemporal pattern of phenology, the physiological response (development) to temperature fluctuations provided better predictions. These results indicate that while researchers rely mostly on temperature data to explain phenological shifts, understanding the non-linear relationship between temperature and development would permit more robust predictions.

Chapter II investigates the temporal changes in sensitivity of phenology to temperature (S_T) using the same data as chapter one. Here, I tested the hypothesis that the phenology of species has become less sensitive to temperature over time, and that this pattern may vary geographically. There was an overall decline in S_T between 1980 and 2013, but geographically this decline was observed along latitudinal but not elevational gradients. To explain this geographic pattern of S_T, I tested three non-mutually exclusive hypotheses: (1) the position of the environmental temperature within the thermal performance curve determines the strength of the response of species to warming, (2) photoperiod has declined after phenological advancements and caused the decline in the thermal response of species, and (3) reduced chilling by winter warming has affected the response of species to spring temperature. I found the strongest support for hypotheses 2 (photoperiod limitation) and 3 (winter warming). These findings reveal that interactions between environmental cues may change the response of species to warming, thereby rendering long-term predictions very challenging.

Chapter III focuses on the use of the thermal performance curve (TPC) for predicting larval development under laboratory and field conditions. Although the average temperature has been used extensively to assess the effect of warming, recent studies have highlighted the relevance of temperature fluctuations in shaping thermal responses. More importantly, there is missing knowledge on the usefulness of TPC in predicting development under natural conditions. I therefore combined laboratory and field experiments in five species of *Sepsis* flies (Diptera: Sepsidae), using distant populations from the temperate region (Europe, Africa and North America), to compare observed development data with theoretical estimates derived from TPC. The predictability of development under fluctuating temperature was better in the laboratory than in the field. Interestingly, accounting for temperatures that fall below a critical minimum improves the predictability of development such that flies not encountering cold conditions show predictable development times whereas flies encountering cold conditions tend to emerge earlier than expected. This study reveals the importance of cold temperatures in

shaping thermal reaction norms, thus providing new insights to improve predictions of the responses of ectotherms to future climate change.

Chapter IV provides the first description of sexual death feigning in a dragonfly species. Death feigning (playing dead) is a widespread behavior in animals, being recorded in mammals, birds, fish, reptiles, amphibians and insects. However, sexual death feigning, with one sex playing dead to avoid the opposite sex, is particularly rare. To date, the only four cases recorded include a species of spider, two species of robber flies and a species of mantis. Although death feigning has been documented in odonates, this thesis is the first record of sexual death feigning in the moorland hawker (*Aeshna juncea*). I suggest that sexual conflict has been the primary driver of the evolution of this behavior due to high levels of male harassment in this species. Since the moorland hawker is widespread in Europe where dragonflies have been studied extensively, it is likely that other cases of sexual death feigning remain undiscovered.

Chapter V presents a commemoration of an important book in odonatology – The Biology of Dragonflies by Tillyard (1917) – and discusses the influence of this book on the field of odonatology, the contributions of the author to the understanding of dragonfly biology and systematics and the scientific advances over the last century. Tillyard has set the foundation for the study of the biology of dragonflies by providing the basic knowledge on their morphology, anatomy and embryology that is still in use today. I divide the history of odonatology into four major periods: Selys era, Tillyard era, Corbet era and the blossoming era (contemporary odonatology). This thesis, as well as many other studies on dragonflies, would not have seen the light of day without Tillyard's (1917) 'The Biology of Dragonflies'; I therefore dedicate a chapter of my thesis to its 100th anniversary.

The results shown in this thesis are particularly important because, unlike plants, ectotherms (cold-blooded animals) have received very little attention in the study of geographic and temporal patterns of phenology. Even though this group of animals encompasses most of the global biodiversity, its members share similar thermal adaptations (thermal performance curve). Consequently, understanding the mechanisms affecting their spatiotemporal pattern of phenology would help us predict their future response and adjust management plans accordingly.

ZUSAMMENFASSUNG

Die Phänologie – der zeitliche Verlauf wiederkehrender biologischer Ereignisse - beeinflusst fast alle Aspekte der Ökologie und Evolution. In den vergangenen Jahrzehnten wurden in einer Vielzahl von Tieren und Pflanzen weltweit phänologische Veränderungen dokumentiert. Phänologische Reaktionen variieren nicht nur zwischen Taxa, sie können auch geographisch variieren, vor allem über klimatische Gradienten wie Breitengrad und Höhenlage. Da phänologische Veränderungen massive Auswirkungen auf den Erhalt von Biodiversität, die menschliche Gesundheit und Wirtschaft haben, ist das Verständnis phänologischer Verschiebungen zur Priorität geworden.

Obwohl phänologische Verschiebungen oft mit Temperaturveränderungen assoziiert werden können, sind die Mechanismen, welche das Ausmass und die geographische Variabilität dieses Verhältnisses beeinflussen bisher nur unzureichend verstanden. Solche Fragen werden idealerweise mit multidisziplinären Forschungsansätzen beantwortet, welche Langzeitbeobachtungen im Freiland mit kontrollierten Laborexperimenten kombinieren. In dieser Arbeit untersuchte ich anhand von Gross- und Kleinlibellen (Odonata), sowie Schwingfliegen (Diptera), wie sich die Temperatur auf die Entwicklung und die Phänologie von Populationen unterschiedlicher Herkunft auswirkt.

Der wesentliche Motivation dieser Arbeit ist es, Kenntnisse über die Faktoren zu liefern, welche die räumlich-zeitliche Phänodynamik beeinflussen, indem sie untersucht wie sich (1) die Phänologie in Nordwesteuropa in den letzten drei Jahrzehnten über die Breiten- und Höhenlage hinweg verändert hat, (2) die thermische Empfindlichkeit der Arten sich zeitlich verändert hat und (3) inwieweit sich thermischen Leistungskurven welche unter Laborbedingungen gemessen wurden ins Freiland übertragen lassen. Darüber hinaus beschreibe ich in meiner Dissertation (4) ein neues Verhalten in Libellen und (5) gedenke dem 100. Jahrestag eines bemerkenswerten Buches der Odonatologie.

In Kapitel I wurde die phänologische Verschiebung der erwachsenen Libellen entlang des Breiten- und Höhengrades mit Langzeitbeobachtungsdaten von 54 Arten aus sechs europäischen Ländern, historischen Temperaturaufzeichnungen und experimentellen

Untersuchungen der thermischen Leistungskurve der Arten analysiert. Geographische Variation in der phänologischen Verschiebung wird erwartet, da das Ausmass der jüngsten Klimaerwärmung zwischen Breiten- und Höhengrad und zwischen Jahreszeiten variiert. Allerdings gibt es wenig Informationen darüber, ob die durchschnittliche Jahrestemperatur, Temperaturschwankung oder die physiologische Antwort auf letzteres am besten bei der Vorhersage der Phänologie hilft. Die phänologische Verschiebung nahm entlang dem Breitengrad zu und mit steigender Erhebung ab. Obwohl die Durchschnittstemperatur einen Teil der Variation des räumlich-zeitlichen Musters der Phänologie erklärte, erlaubt die physiologische Antwort (Entwicklung) zur Temperaturschwankung bessere Vorhersagen. Diese Ergebnisse deuten darauf hin, dass, während Forscher sich vor allem auf Temperaturdaten verlassen um phänologische Verschiebungen zu erklären, das Verständnis der nicht-linearen Beziehung zwischen Temperatur und Entwicklung bessere Vorhersagen erlaubt.

Kapitel II untersucht zeitliche Veränderungen der Temperaturempfindlichkeit der Phänologie(ST) unter Verwendung der gleichen Daten wie in Kapitel eins. Hier prüfe ich die Hypothese, dass die speziesspezifische Temperaturempfindlichkeit der abnimmt und ob sich dieses Muster geographisch ändert. Zwischen 1980 und 2013 konnte ein allgemeiner Rückgang in STentlang des geographischen Breitengrades aber nicht mit steigender Höhe nachgewiesen werden. Um diesen Befund zu erklären, wurden 3 Hypothesen getestet: (1) Die Lage der Umgebungstemperatur relativ zur thermischen Leistungskurve könnte die Stärke der Reaktion einer Spezies auf eine Erwärmung bestimmen, (2) aufgrund von verfrühtem Eintreten der Phänologie ist die Photoperiode verkürzt, was eine Reduktion der temperaturabhängigen Reaktion der Spezies mit sich ziehen könnte und (3) wärmere Winter könnten die Reaktion der Spezies auf Frühjahrstemperaturen beeinflussen. Meine Ergebnisse unterstützen die Hypothesen 2 (verkürzte Photoperiode) und 3 (wärmere Winter) als Beste, um die räumlich-zeitliche Variation der ST erklären. Diese Ergebnisse zeigen, dass eine Wechselwirkung von Umweltreizen die Reaktion von Spezies auf Erwärmung verändern kann, was Langzeitvorhersagen sehr anspruchsvolle macht.

Kapitel III konzentriert sich auf die Verwendung der thermischen Leistungskurve (TPC) um die Larvalentwicklung unter Labor-und Feldbedingungen vorherzusagen. Bislang wurde die durchschnittliche Temperatur verwendet um die Wirkung einer Temperaturerhöhung zu beurteilen, jüngste Studien heben allerdings die Bedeutung von Temperaturschwankungen für temperaturabhängige Reaktionen hervor. Des Weiteren ist noch vieles unbekannt über die

Nützlichkeit der TPC für die Vorhersage der Entwicklung unter natürlichen Bedingungen. In Labor- und Feldversuchen wurden für fünf Sepsidenarten (Diptera: Sepsidae)und jeweils weitentfernte Populationen aus den gemäßigten Zonen Europas, Afrikas und Nordamerikas Entwicklungsdaten mit theoretischen TPC-Schätzwerten verglichen. Die Entwicklung unter schwankenden Temperaturen war im Labor besser vorhersehbar als im Feld. Interessanterweisereduzieren Temperaturen unterhalb des kritischen Minimums die Vorhersagbarkeit der Entwicklungsdauer so, dass Fliegen, , die auf kalte Bedingungen stoßen, früher schlüpfen als erwartet, während sich die Entwicklungsdauer von Fliegen, die nicht unter kalten Bedingungen aufwachsen, gut schätzbar ist. Diese Studie zeigt den Einfluss kalter Temperaturen auf die thermische Reaktionsnorm und bietet damit neue Ansätze für eine Verbesserung der Vorhersage der Reaktion von Ektothermen auf künftigen Klimawandel.

Kapitel IV beschreibt zum ersten Mal das Auftreten einer Schreckstarre in sexualem Kontext in einer Libellenart. Die Schreckstarre ist ein weitverbreitetes Verhalten im Tierreich und kann bei Säugetieren, Vögeln, Fischen, Reptilien, Amphibien und Insekten beobachtet werden. Die Schreckstarre als Verhalten um Stress durch das andere Geschlecht zu entgehen ist allerdings mit nur vier bekannten Fällen sehr selten. Dieses Verhalten konnte bisher in einer Spinnenart, zwei Arten von Räuberfliegen und einer Mantisspezies dokumentiert werden. Es ist bekannt, dass sich gewisse Libellen Arten totstellen können, aber in sexuelem Kontext, wie hier in der Torf-Mosaikjungfer (*Aeshna juncea*), wwurde dieses Verhalten noch nie beobachtet. Ich schlage sexuellen Konflikt als Hauptgrund fürdie Evolution dieses Verhaltens vor, da die Belästigung von Weibchen durch männliche Artgenossen in dieser Art sehr häufig ist.

Kapitel V gedenkt einem wichtigen Buch in der Odonatologie - The Biology of Dragonflies von Tillyard (1917) - und diskutiert den Einfluss des Buches auf dem Gebiet der Odonatologie, die Beiträge des Autors zur Biologie und Systematik der Libellen und den wissenschaftlichen Fortschrittdes letzten Jahrhunderts. Tillyard hatte das Fundament der Biologie der Libellen gelegt, indem sie Grundkenntnisse über die Morphologie, Anatomie und Embryologie niederschrieb, welche heute noch verwendet werden. Ich unterteile die Geschichte der Odonatologie in vier große Perioden: Selys Ära, Tillyard Ära, Corbet Ära und die blühende Ära (zeitgenössische Odonatologie). Diese Dissertation, sowie auch viele andere Studien über Libellen, wären ohne dieses Buch nicht geschrieben worden. Aus diesem Grund widme ich ein Kapitel meiner Arbeitdem 100. Jahrestag dem Buch “The Biology of Dragonflies“ von Tillyard (1917).

Die Ergebnisse der Studien in dieser Dissertation sind von besonderer Wichtigkeit, weil im Gegensatz zu Pflanzen, welche weitgehend in der die Grundlage zur Erforschung geographischer und zeitlicher Muster der Phänologie verwendet wurden, haben Ektothermen (Kaltblüter), welche einen Grossteil der globalen Fauna ausmachen, wenig Aufmerksamkeit erhalten. Darüber hinaus teilen sie sich ein ähnliche thermische Anpassungen (z.B. die thermische Leistungskurve) und deshalb kann das Verständnis der Mechanismen, welche räumlich-zeitliche Muster der Phänologie beeinflussen, uns helfen, zukünftige Reaktion vorherzusagen und entsprechende Managementpläne zu machen.

GENERAL INTRODUCTION

Phenology is the timing of recurrent biological events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species (Lieth 2013). The term phenology was first introduced in 1853 by the Belgian botanist Charles François Antoine Morren (1807-1858) (Puppi 2007). One of the first detailed phenological data sets was collected by Konrad Gessner who worked in Zurich during the first half of the 16th century and described the timing of leafing, flowering and fructification of 1250 tree, shrub and herb species in his De stirpium collection (published posthumously in 1587). The science of phenology underwent an important development in the 17th century with the contributions of Robert Marsham, Carl Linnaeus and René-Antoine Ferchault de Réaumur. Nowadays, there are phenological monitoring networks in different continents with multidecadal databases for several species of animals and plants (Tang et al. 2016).

In temperate region, species have evolved specific phenologies that are adapted to local environment. However, the phenology of a given species changes from one year to another depending on weather conditions (temperature, rainfall, etc.). For example, temperature is one of the main factors that affects development and thus phenology (Honěk and Kocourek 1990, Adams et al. 2001). In warm years, the timing of a certain event such as flowering starts earlier (Price and Waser 1998). Rainfall is also an important factor influencing the phenology of organisms. For instance, for species that depend on water to reproduce such as some frogs, egg laying has to be carried out after rain (Bourne et al. 2001).

Although there are usually year-to-year fluctuations of the timing of certain phenological events, studies have detected a systematic and gradual pattern across a range of species during the past few decades. Advancement in the phenology of species as a result of global warming is a widespread phenomenon (Parmesan 2007, Post 2013). It is a central topic in ecology due to its economical, human health and ecological issues (Ziska et al. 2011, Mozell and Thach 2014). In many taxa, the recent increase in temperature has affected the timing of life history traits (Sherry et al. 2007, Benard 2015), and induced drastic changes in the population dynamics of top predators and even extinctions due to mistiming (Both et al. 2006, Cahill et al. 2012). The globally reported

pattern in different ecosystems is an advancement of the phenology over time (Parmesan 2007). However, the long term change in temperature is not uniform across geographic gradients such as latitude and elevation (La Sorte et al. 2014), and consequently, phenological responses vary accordingly (Frei et al. 2014). We have a poor understanding of how climate change affects biotic responses to changing thermal regimes along the dominant geographic gradients.

Forrest and Miller-Rushing (2010) said "phenology affects nearly all aspects of ecology and evolution. Virtually all biological phenomena—from individual physiology to interspecific relationships to global nutrient fluxes—have annual cycles and are influenced by the timing of abiotic events." The importance of studying phenology lies in its implications in population dynamics, evolution, human health and ecosystem functioning (Both et al. 2006, Franks et al. 2007, Jepsen et al. 2011, Ziska et al. 2011). For instance, the length of the ragweed pollen season has increased substantially in North America following change in climate, which has resulted in longer exposure to seasonal allergens for people and subsequent effects on public health (Ziska et al. 2011). Another example of potential consequence of phenological change is the mismatch that can occur when the timing of the availability of a resource does not meet the timing of the demand for this resource. Both et al. (2006) have shown that 90% of the pied flycatcher populations in the Netherlands went extinct because the peak of caterpillar abundance for nestlings shifted earlier and the timing of migration of the bird did not change. Therefore, the understanding of factors affecting the magnitude of phenological is important to predict future potential alteration of biotic interactions that may disrupt ecosystem functioning.

In temperate regions, phenology changes considerably along latitude and elevation (Fielding et al. 1999). The higher the elevation or latitude, the later the phenological event. One of the main factors that varies along both gradients is temperature. However, thermal variation is steeper along the elevational gradient than the latitudinal gradient: the decrease in temperature with 1000 m elevation is roughly equivalent to the decrease occurring over 1000 km latitude (about 6°C in both cases) (Colwell et al. 2008). This makes the changes in phenology per unit of distance more pronounced along elevation than latitude. What makes the dynamics of phenology more difficult to predict is that historical changes in temperature have not been homogeneous on the two gradients: northern latitudes have warmed relatively fast, whereas higher elevations have warmed less than low elevations (Menéndez et al. 2014). Although some studies on the effects of climate change on life history traits have been carried out on both gradients (Parmesan 2007), no

study has compared the dynamics of phenology in both gradients using the same taxa and timescale.

Odonates (dragonflies and damselflies) are good study organisms to address questions on phenology and its spatiotemporal dynamics. First, they are conspicuous and easy to identify in the field (Cordero-Rivera and Stoks 2008). This has allowed scientists and amateurs to carry out repeated observations of adults over decades in natural habitats. Second, they are widespread and occur in all continents except Antarctica (Corbet 1999). Some species have large latitudinal and elevational ranges, which is suitable to carry out studies on life history variation and adaptation to local environments (Stoks and McPeek 2003, De Block and Stoks 2004, Stoks et al. 2006, De Block et al. 2008). Third, they are ectotherms and thus their physiology is highly depended on temperature (Shama et al. 2011, Nilsson-Örtman et al. 2012), which make them excellent candidates to track the potential effects of warming on phenology (Hassall et al. 2007). Finally, they have a complex life cycle with an aquatic larval stage and a terrestrial adult stage (Stoks and Córdoba-Aguilar 2012). This means that odonates interact with both aquatic and terrestrial organisms, thus phenological shift may lead to potential mistiming between trophic levels (Both et al. 2006) with ecological consequences on both ecosystems.

Objectives

Although phenology has been extensively studied during the past two decades, we still have little information on its spatiotemporal dynamics across geographic gradients such as latitude and elevation. Furthermore, many studies have focused on the effect of average temperature on phenology, paying little attention on the potential implication of fluctuation of temperature. Moreover, in order to reliably predict future changes of phenology, it is important to determine whether the thermal relationship of phenological shift is constant or not, and which factors might influence the increase of decrease of the phenological response.

In this thesis, I address three questions related to the understanding of spatiotemporal dynamics of phenology (**Chapter I-III**), I present a discovery on the behavior of adult odonates (**Chapter IV**) and I commemorate the hundred anniversary of a noteworthy book for odonatology (**Chapter V**).

Chapter I describes the phenological shift of odonates during the last three decades across a latitudinal gradient in Europe and an elevational gradient in Switzerland and assesses the main factors that explain the geographic pattern. Three types of data are used: (1) long-term observation

data of adult odonates (54 species) from six European countries (Switzerland, Germany, Belgium, Netherlands, UK and Sweden) during 1980-2013, (2) temperature data collected from meteorological stations across the six countries, (3) and ecophysiological data on experimentally estimated thermal performance curves of nine species of odonates.

Chapter II uses the same data as **Chapter I** to assess changes in sensitivity of phenology to temperature over the three last decades. It compares thermal sensitivity across latitudinal and elevational gradient. The implication of factors that play a potential role in the dynamics of thermal sensitivity are explored. Results provide insight into the reliability of the prediction of global warming effects in ectotherms.

Chapter III addresses three main questions in thermal ecology of temperate insects, that is, (1) do thermal performance curves changes with latitude and elevation? (2) What are the effects of fluctuation of temperature on life history traits? Would rate summation derived from the thermal performance curve give reliable estimates of the effects of fluctuation of temperature? These questions are of primary importance because they reveal potential adaptive (or not) mechanisms across geographic gradients; highlight whether there is a risk in overlooking fluctuation of temperature in the estimation of thermal responses of ectotherms, and show the usefulness of thermal performance curve (estimated with constant temperatures) to predict responses to fluctuation of temperature.

Chapter IV describes a new behavior in dragonflies in which the female uses tonic immobility (death feigning) to escape male harassment. This behavioral study was conducted on the moorland hawker (*Aeshna juncea*) in Switzerland, mainly in two high elevation ponds. This finding opens the question of whether the scarcity of sexual death feigning in nature is rare or just an artifact.

Finally, **Chapter V** commemorates the 100th anniversary of the Biology of Dragonflies by Tillyard 1917, a key book that played an important role in the establishment of odonatology. In this chapter, I present an overview on the book, the biography and the scientific contribution contributions of the authors to the taxonomy and biology of dragonflies, the importance of the book to biologists and non-scientists, a short history of the field and the most important ecological, biological and behavioral aspects that we have learned during the last century.

With these four chapters, this thesis not only fills in essential gaps of knowledge on the spatiotemporal dynamics of phenology in temperate region, but also sets the ground for relevant questions to address in the future. Moreover, unlike the available literature on phenological

dynamics across latitude and elevation which deals mainly with plants, this work has been done on ectotherms and thus provides new information and perspectives for the implications and consequences of climate change on variation in the phenology of species. Furthermore, this thesis improves our understanding of the influence of temperature on ectotherms' phenology, and specifically of the mechanistic processes behind the thermal effects and the potential temporal changes of the latter. Ectotherms have similar thermal adaptations and thus the findings presented and discussed in this thesis could be applicable for many ectotherms. Last but not least, the discovery of a new behavior in a common species of odonates should raise the awareness of scientists and amateurs on the overwhelming natural history information that is still waiting to be discovered.

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CHAPTER I

Geographic gradients in climate change response explained by non-linear thermal-performance curves

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Climate change has caused widespread shifts in phenology – or the timing of life cycle events – and these can have important consequences for the performance of individuals and the composition of ecological communities. However, little is known of the mechanistic connection between the physiology of individuals and the phenology of populations, although this connection could help predict future climate impacts. We combined experiments and long-term monitoring data on dragonflies and damselflies to discover that geographic variation in response to climate change arises from the non-linear shape of the relationship between development and temperature, the so-called temperature-performance curve (TPC). Across 54 European odonate species, field data revealed a shift in the adult flight season toward earlier dates between 1980 and 2013; this remained relatively constant with latitude but decreased with elevation. The geographic pattern of climate response was predicted accurately with the changes in late spring and early summer conditions where development is highest. The spatiotemporal dynamics of phenology was further predicted by projecting long-term trends in both mean and daily variation of temperature measured at weather stations onto TPCs estimated in the laboratory. The convex downward shape of the ascending portion of the TPC means that warming temperatures induce a greater phenological response in warmer regions and that increasing temperature variation accelerates phenology. Hence, geographic variation in climate impacts is predictable by scaling up from the physiological effects of temperature on individuals.

Keywords: Phenology, odonates, dragonflies, warming, fluctuation of temperature, Jensen's inequality

1.1. Introduction

There is considerable geographic variation in the responses of plants and animals to climate change (Primack et al. 2009, Phillimore et al. 2010, Hurlbert and Liang 2012). The causes of geographic variation are largely unknown but could be important for predicting and managing future climate impacts (Thomas et al. 2004, Loarie et al. 2009, Primack et al. 2009, Urban et al. 2016). One obvious explanation − that the responses of organisms simply reflect the extent of temperature change − is not supported by studies observing weak relationships between the spatial pattern of climate warming and the magnitude of the biotic response (Rubolini et al. 2007).

Determining how species' temporal niches change due to climate warming is crucial, considering the rapid global change (Lovejoy and Hannah 2005). In many taxa, a shift in the temporal niche induced by warmer temperature has led to changes in life history traits (Sherry et al. 2007, Benard 2015), biotic interactions (Janssens et al. 2015) and population dynamics of top predators and even extinctions due to mistiming (Both et al. 2006, Cahill et al. 2012). The globally reported pattern in different ecosystems is an advancement of phenology over time (Parmesan 2007). However, long term increase in temperature can change along geographic gradients (e.g. latitude and elevation) (La Sorte et al. 2014), and consequently, phenological responses vary accordingly (Mazaris et al. 2013, Diamond et al. 2014, Frei et al. 2014). So far, we have a poor understanding of how global warming affected thermal regimes in the dominant geographic gradients and how these changes would affect the physiology of species and alter life history traits.

Many organisms, especially ectotherms, which represent the highest diversity on earth (Wilson 1992), respond non-linearly to temperature (Huey and Stevenson 1979). Although this finding was reported a few decades ago, few studies have made the link between this non-linear relationship and the geographic pattern of the response to global warming (Deutsch et al. 2008). No study has investigated the role of this non-linearity in shaping the response of species to changes in thermal regimes (including both the mean and variation in temperature) along the dominant terrestrial gradients, latitude and elevation. Here, we present such an analysis of odonates (dragonflies and damselflies), widespread top-predator aquatic insects with complex life cycle (Stoks and Córdoba-Aguilar 2012), which occur on all continents (except Antarctica) (Corbet 1999). The adults of these insects dominate freshwater ecosystems in spring and summer, and constitute a considerable proportion of the diet of many birds and frogs (Graveland 1996, Jancowski and Orchard 2013). Phenological response of odonates to climate change in some parts

of Western Europe have been reported (Hassall et al. 2007, Dingemanse and Kalkman 2008), but no conclusive account on the geographic dynamics can be made since these studies have been conducted at different time scales, with different sets of species and within a restricted geographic area. In this study, we aim to compare the temporal pattern of phenology of 54 species of odonates (Table S1) across latitude and elevation by using long term observation data from six Western European countries (from 46° to 64° latitude including the Swiss Alps) carried out during 1980-2013, a period during which temperatures increased tremendously (IPCC 2014).

Although annual temperature has been widely used to predict life history changes in ectotherms (Deutsch et al. 2008), this measure masks considerable diurnal and seasonal variation in temperature that are relevant for life history events. To investigate the effect of warming on phenological shifts, it is crucial to identify the time window that species are thermally most sensitive to (Trudgill et al. 2005). For instance, in odonates, species emerge in spring and summer and thus thermal cues during these seasons are important for the timing of emergence (Dingemanse and Kalkman 2008). Furthermore, unlike the start of the flight season, which seems to globally shift towards earlier dates, the late phase could show different magnitude and direction of change (Hassall et al. 2007). Although factors that affect the start of the flight season should be mainly related to development of larvae, the end of the season could be related to life span, which depends mainly on environmental conditions (Sherratt et al. 2010).

In this study, we investigate the temporal pattern of phenology using long-term observation data to explain the direction and the magnitude of the phenological shift across geographic gradients using both temperature data and physiological rates (development data) (Fig. 1). We addressed the following questions: (1) what is the role of average and fluctuation of temperature in explaining the geographic pattern of phenological shift? (2) how change in development explains phenological shifts?

1.2. Methods

1.2.1. Dataset

We assembled odonate atlas data covering the period 1980 - 2013, collected in northcentral Europe from 46 °N to 69 °N and -5 m to 2750 m elevation (Fig. 2A, B). The countries and data sources, in order from south to north, were Switzerland (Centre Suisse de Cartographie de la Faune), Germany (Arbeitskreis zum Schutz und zur Kartierung der Libellen in Nordrhein-Westfalen),

Belgium (Instituut voor Natuuren Bosonderzoek), the Netherlands (EIS Kenniscentrum Insecten en andere ongewervelden), United Kingdom (British Dragonfly Society), and Sweden (the website http://svalan.artdata.slu.se/bugs/). The dataset includes ~2.53 million observations of adult odonates of 54 species (Table S1). The resolution of the data was reduced to 1° latitude/longitude.

1.2.2. Data preparation

For each combination of year, species and site (grid), we characterized the phenology of adults by the Julian date at which 15%, 50%, and 85% of observations were recorded. These quantiles describe the timing and duration of the flight period, and are less sensitive to sampling effort than the dates of first and last observation (Miller-Rushing et al. 2008). A species was included if there were ≥ 8 individuals observed in ≥ 15 years extending over a range of ≥ 25 years. Monthly mean and daily minimum/maximum air temperatures were obtained from weather stations in Switzerland (63 stations from http://www.meteosuisse.admin.ch/), Germany (41 stations from http://www.dwd.de/), Netherlands (1 station from http://www.knmi.nl/home), Belgium (3 stations from http://www.meteo.be/), United Kingdom (21 stations from http://www.metoffice.gov.uk/) and Sweden (29 stations from http://luftwebb.smhi.se/). These temperature data were interpolated using kriging such that each grid had a monthly temperature record. Water temperature is closely correlated with air temperature, at least over a regional spatial scale (Piccolroaz et al. 2013).

1.2.3. Analyses

Geographic variation in phenology across latitude and elevation was first analyzed with a simple linear model then with a mixed effects model (LME) including species and grid (site) as random effects. Temporal phenological changes were estimated with an LME with date of the three quantiles (phenophases; 15%, 50% and 85%) as the response, species and grid as random effects, and latitude, elevation and year as fixed effects. The overall change in timing was assessed with a reduced version of this model containing only year as fixed effects and species as a random effect. All models were implemented in the R package lme4 (Bates et al. 2015).

Whenever temperature contributed to an analysis, we used values from the temperature-sensitive period (TSP), defined as the time window over which temperature most strongly influenced phenology. We tested for monthly average temperature of all months excluding winter (December-February) for the focal (March-July) and previous year (March-November). The TSP

was the time window which gave the lowest Akaike Information Criterion (AIC). Long-term changes in average temperature (T_{mean}) and temperature range (T_{range}) for time windows were assessed with LMEs using year, latitude and elevation as fixed effects and species and site as random effects.

1.2.4. Larval rearing

We followed the procedure used by (Śniegula and Johansson 2010) to obtain eggs from damselflies and endophytic dragonflies and that used by (Khelifa et al. 2012) to collect eggs from exophytic dragonflies. Species, sampling sites, and number of females collected are presented in Table S2. Eggs were collected from the different ponds in Switzerland during the reproductive seasons of 2014 and 2015, and moved to the laboratory where they were placed in plastic containers (20 x 10 x 10 cm) containing old non-chlorinated tap water and maintained at 21°C with a photoperiod of 14:10 light:dark (L:D) inside a climate chamber. For species with diapausing eggs, we simulated winter conditions by putting eggs in the dark by covering them with black plastic bag for a day then in the fridge at 5°C for 8 days to make sure that embryos experience low temperatures and stop diapause (Corbet 1956). To imitate the arrival of spring conditions, we kept eggs in the dark but increased temperature to 15°C for three days, then we induced daylight by removing the black plastic bag and raised temperature to 21°C. Eggs were daily checked for hatching. Due to the synchronous hatching, larvae were placed at the same time in 0.3-l cups floating on a water bath by polystyrene.

1.2.5. Estimation of the thermal performance curve

Twelve larvae from each female were randomly selected, placed individually in 0.3-l cups in six temperature treatments (18, 21, 24, 27, 30 and 33°C), and fed everyday with brine shrimp, *Artemia* sp., nauplii ad libitum. The head width of each larva was measured every 4 days using a microscope. For zygopterans, a couple piece of grass were provided as perch. Zygopterans were reared up to their emergence, but anisopterans were investigated up to 60 days because they take several months to reach the adult stage.

Based on the six temperature treatments, thermal performance curves (TPCs) were fitted with the O'Neill function (Spain 1982) which estimates three of the four components of the response to temperature (T_{opt}, T_{max}, Q_{10}) (Suhling et al. 2015). The model assumes that the growth increases with temperature with a coefficient Q10 within the range of temperatures prior to the

optimal temperature (T_{opt}) where the growth peaks (k), and decreases abruptly afterwards until the maximum temperature (T_{max}) where growth is null.

$$\Phi(T) = k\left(\frac{T_{max}-T}{T_{\max -}\ T_{opt}}\right)^{p} \exp\left(\frac{p(T-T_{opt})}{T_{max}-T_{opt}}\right)$$

where $p = \frac{1}{400} J^2 \left(1 + \sqrt{1 + \frac{40}{J}}\right)^2$ and $J = (Q_{10} - 1)\left(T - T_{opt}\right)$, φ(T) the respective mean growth rate from experimental data, k is the maximum frowth rate, and T is the ambient temperature.

Using the fitted function, we predicted average growth (G_{mean}) as the response of the average temperature of the TSP (Fig. 3a) and mid-point growth (G_{mid}) as the middle distance between the maximum growth (response to diurnal maximum monthly temperature) and minimum growth (response to diurnal minimum monthly temperature) (Fig. 3b), thus accounting for fluctuation of temperature. We then used average temperature, G_{mean} and G_{mid} as predictors of long-term change in phenology in separate LMEs in which the dependent variable was the observed date of the three quantiles with species and sites as random effects. We assume that TPCs do not change dramatically with latitude and elevation because laboratory experiment on the TPC of three species (*Ischnura elegans*, *Enallagma cyathigerum* and *Aeshna juncea*) on populations at low and high elevation in the Swiss Alps showed that TPC do not vary (Khelifa R. unpublished data).

Temperature-dependent model was fitted for the three quantiles because environmental temperature can affect the early, the middle and the late phase of phenology, whereas growth rate-dependent models were fitted only for the early phase because the middle and late phase are affected by other factors than development (such as adult lifespan and terrestrial environmental conditions). The model that best predicted the geographic variation in phenological change among species was that with the lowest AIC value.

1.3. Results

1.3.1. Geographic cline of phenology

Data of all years were pooled together to assess how phenological distributions change with latitude and elevation. There was a geographic cline of the phenology of odonates across latitude and elevation (Fig. 4, Table 1). On average (excluding species), the simple linear model showed that the duration of the flight season – the period between the 15% and 85% quantiles – declined

across latitude by 2.41 $d \cdot °lat^{-1}$ and across elevation by 0.02 $d \cdot m^{-1}$. Based on the duration of flight season, the lapse rate between latitude and elevation was 120.5 m elevation per degree latitude. The decline of duration of the season in latitude resulted from a spatial delay of the early phase by 1.64 $d \cdot °^{-1}$ and an advancement of the late phase by 0.77 $d \cdot °^{-1}$. Similarly, across elevation the shortening of the flight season resulted from a delay of the early phase by 0.02 $d \cdot m^{-1}$ and an advancement of the late phase by 0.003 $d \cdot m^{-1}$. The mixed effects model (including species and grid as random effects) showed similar patterns but with slightly different extents. Additionally, there was significant interspecific variation in the duration of the flight season (random species intercept: $\chi^2 = 5362.1$, $P < 0.0001$, Table 2), and in the latitudinal and elevational cline (heterogeneity among species in slopes against latitude and elevation: $\chi^2 = 1079.9$, $P < 0.0001$, Table 2).

1.3.2. Temporal pattern of phenological shift

The temporal pattern of the phenological distribution of species was assessed by regressing phenological phases and season duration against years (1980-2013). On average, phenology has advanced by 0.16 (95%CI: 0.15 – 0.16) $d \cdot yr^{-1}$ for the early phase of the flight period (the date by which the first 15% of individuals were recorded), by 0.06 [0.05 – 0.07] $d \cdot yr^{-1}$ for the middle phase (median date), and it shifted towards later dates by 0.04 [0.03 – 0.05] $d \cdot yr^{-1}$ for the late phase (85% quantile) (Fig. 5). The duration of the flight season has increased by an average of 0.25 [0.25–0.26] $d \cdot yr^{-1}$ over the 34 years.

The overall pattern of phenological advancement masks considerable geographic variation (Fig. 5). The general increase in the duration of the flight season was stronger at high latitude than at low latitude, revealing that the adult season increased more in Sweden than in lowland Switzerland; however, the increase was lowest at high elevation, in the Swiss Alps (Fig. 6; Table 3). This geographic variation of the temporal pattern of the duration of the adult season resulted from differential changes in the different quantiles of the phenological distribution. In fact, the magnitude of the phenological shift for the early and middle phase remained constant across latitude and decreased across elevation (Fig. 5). The strength of the shift of the late phase increased with latitude but remained relatively constant with elevation (Fig. 5; Table 4).

1.3.3. Linking temperature to phenology

There was a general warming of all months of the year (excluding December-February) during 1980-2013. The month that has shown the most overall warming is April (0.060°C·yr^{-1} [95%CI: 0.054-0.064]). Lower warming was recorded for March-July and September (0.029-0.033 °C·yr^{-1}), whereas the least warming months were August (0.020 °C·yr^{-1}) and October (0.022 °C·yr^{-1}) (Table S3).

We regressed phenology against the average temperature of all potential temperature windows (months) that could explain the phenological shift of the early, middle and late phase during 1980-2013. Late spring and early summer (May and June) temperatures of the focal year were the best predictors for the early and middle phases, whereas late summer and early autumn were the best time windows for the late phase (Table S4). Sensitivity of the early and middle phase were negative; that is, an increase of temperature led to earlier emergence. However, sensitivity of the late phase was positive, which means that an increase of temperature led to an extended flight season.

1.3.4. Linking development to phenology

Thermal performance curves (TPC) of nine species fitted with the O'Neil function are presented in Figure 7. Using TPCs, we predicted the changes in growth rate in response to changes in average temperature (average growth rate: G_{mean}) and diurnal fluctuation of temperature (mid-growth rate: G_{mid}) of the time window that best explained phenological change of the early phase of the nine species (Fig. 8). G_{mid} was larger than G_{mean} (t-test: t = 36.878, df = 16347, p-value < 0.0001), showing that fluctuation of temperature had a positive effect on growth rate.

Here we try to explain the temporal pattern of the early phase with three independent LME models testing for the importance of three predictors separately: average temperature (T_{mean}), G_{mean} and G_{mid}. We did not use these models for the middle and late phase because the latter are influenced by other terrestrial abiotic and biotic factors that are not related to the larval aquatic stage. We compared the three models to explain the spatiotemporal pattern of the early phase using AIC. G_{mid} was much more strongly supported by the data than either of the others (values of Akaike information criterion, AIC for G_{mid} =821.3×10^3, for T_{mean} = 822.2×10^3, and for G_{mean} = 822.9×10^3, respectively) (Fig. 9). Overall, unlike average temperature (Table 5), the temporal pattern of temperature range (Table 6) matched that of phenological changes of the early phase. The combined effects of average and range of temperature produced changes in growth rates (Fig. 9) that fitted the phenological shift of the early phase (Fig. 8).

1.4. Discussion

Our study unraveled the phenological changes of odonates in northcentral Europe across latitude and elevation and reveals the implication of climate warming. We found (1) an overall phenological shift which resulted in an increase in the duration of the phenological season, (2) geographic variation in the magnitude of the phenological shift -- declining across elevation for the early phase and increasing across latitude for the late phase, and (3) a strong relationship between temperatures of late spring and early summer and the temporal patterns of phenology.

The geographic cline in phenology across latitude and elevation is a widespread pattern in the temperate zone (Tauber et al. 1986). Due to the shorter warm season at high latitude and elevation, species emerge later in the north and high elevation than the south and low elevation (Corbet 1999). In our study of odonates, the duration of the flight season showed a stronger decrease across elevation than across latitude with a lapse rate of 120.5 m elevation per degree latitude. This is relatively comparable to the lapse rate between latitude and elevation of ≈140 m elevation per degree latitude across a European transect (Halbritter et al. 2013). However, the change in the flight season is mainly due to a strong delay of the early phase from low to high latitude and elevation. This is consistent with the climatic conditions at higher latitudes and elevations where the frozen ponds start to melt in early summer while they melt in the spring at low latitudes and elevations (Post 2013).

The phenological advancement of the adult season of north-central European odonates is similar in terms of direction to many other species (Parmesan 2007). The overall phenological shift recorded in our study during 1980-2013 for the early phase (0.16 $d \cdot yr^{-1}$) was similar to that recorded for British dragonflies during 1960-2004 (Hassall et al. 2007). Moreover, the direction of the phenological shift of the early phase was towards earlier dates and that of the late phase was towards later dates, which led to the increase of the flight season of odonates. Although the advancement of the early phase was clearly due to earlier emergence of adults, the stretching of the late phase could be the result of an increase in the lifespan of individuals (following an improvement of environmental conditions), an augmentation in the number of generations produced per flight season, and a potential decrease in the synchronicity of emergence (Corbet 1999).

The phenological shift presented a geographic pattern across latitude and elevation. On average, the magnitude of the shift of the early phase increased significantly across latitude, but

declined across elevation. It is known that climate warming increases towards the north (European Environment Agency 2015) but declines with elevation (Menéndez et al. 2014). By linking the temporal pattern of temperature data to phenological changes, we revealed that species were most sensitive to late spring and early summer temperatures of the focal year. In fact, larvae during this period develop and use environmental cues like temperature and photoperiod to time their emergence, which explains their thermally close relationship with this time window. Furthermore, the fact that the stretching of late phenological phase was best explained by warming of late summer and early autumn temperatures suggests that (1) a cohort of larvae might have developed fast during summer and emerged as the second generation of the year at low latitudes and elevations (Mahdjoub et al. 2015, Khelifa 2017), but at high latitude where the late phase extension was even higher, the extended late sason could be the result of longer life span due to improvement of weather conditions (Sherratt et al. 2010).

Interestingly, we found that the estimation of growth rate based on average and fluctuation of temperature was a better predictor of phenology than temperature. We looked at the effect of warming from a physiological point of view by assessing growth rate of nine species. First, temperature performance curves (TPCs) of all species was typical of ectotherms (Deutsch et al. 2008) with a gradual non-linear increase of performance (growth rate) until the optimal temperature (around 27°C) followed by an abrupt decline. We estimated two measures of growth rate: G_{mean} which is the response to average temperature and G_{mid} which accounts for both average and diurnal fluctuation of temperature. We found that the early phase of phenology was best predicted by $G_{mid.}$ This shows that temperature data alone are insufficient to reliably predict year-to-year variation in phenology and that the thermal physiological response are crucial to achieve good predictability. The fact that the early phase, which is correlated to emergence, was best predicted by G_{mid} means that larval development responded to fluctuation of temperature such that individuals emerged earlier than expected from the average temperature alone. This could be explained theoretically by Jensen's Inequality, that is, the fluctuation of temperature induces higher developmental response than that of the average temperature because the relationship between temperature and development is usually nonlinear (Ragland and Kingsolver 2008, Vasseur et al. 2014). These findings highlight the importance of variation in temperature in depicting the actual physiological rates of ectotherms and accurately predicting development and phenology.

This study is the first to document large-scale phenological changes of odonates across latitude and elevation. Our results showed that the phenology shifted differently across these two geographic gradients, and this spatiotemporal pattern is explained by warming of temperature conditions in late spring and early summer; prior to emergence. We demonstrated that while the shape of the phenological distribution did not change at high elevation, it changed considerably and differently at low and high latitude. This could have implications on the intra-guild temporal segregation of species which might affect intra- and interspecific interactions such as competition, predation and cannibalism (Pierce et al. 1985, Van Buskirk 1989). Furthermore, our study demonstrates that thermal performance curves can be used to project phenological change from data on the mean and variation in temperature, and the results accurately predict geographic trends in climate response, better than temperature. This goes in line with studies observing weak relationships between the spatial pattern of climate warming and the magnitude of the biotic response (Rubolini et al. 2007, Primack et al. 2009), thus confirming the importance of the non-linear relationship between temperature and physiological rates in reliably predicting phenology. These findings are relevant for all ectotherms because they share similar non-linear TPCs to odonates.

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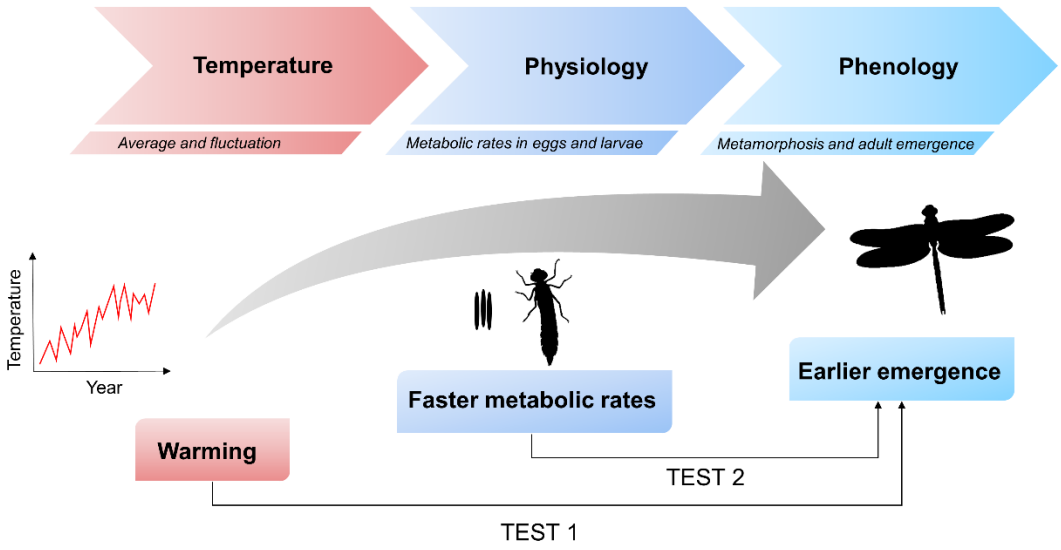

Figure 1. Relationship between temperature, physiology and phenology. Phenology of adults is influenced by physiological processes (metabolic rates) that are strongly dependent on temperature. So, temperature warming leads to faster metabolic rates at the egg and larval stage which fosters development and leads to earlier emergence of adults. Test 1 (temperature-phenology relationship) and Test 2 (development-phenology relationship) were performed in this study.

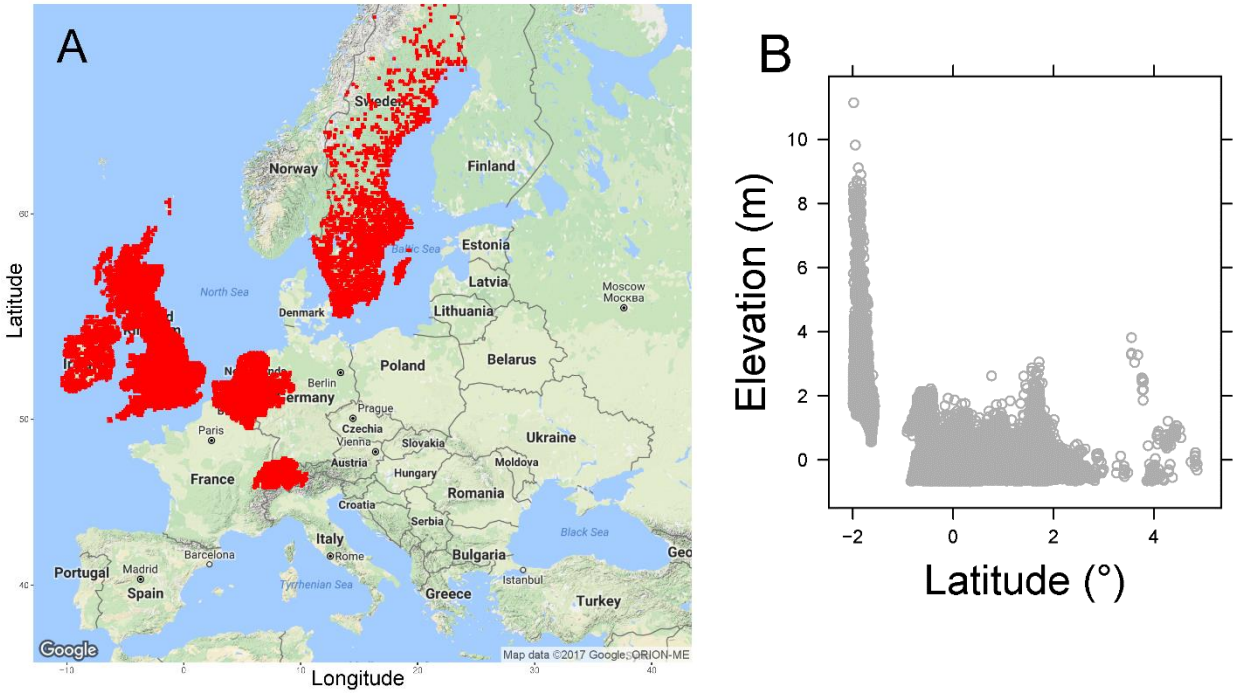

Figure 2. A. Study area in northcentral Europe. Red dots are observation sites of adult odonates. **B**. Geographic distribution of observation sites across standardized latitude and elevation.

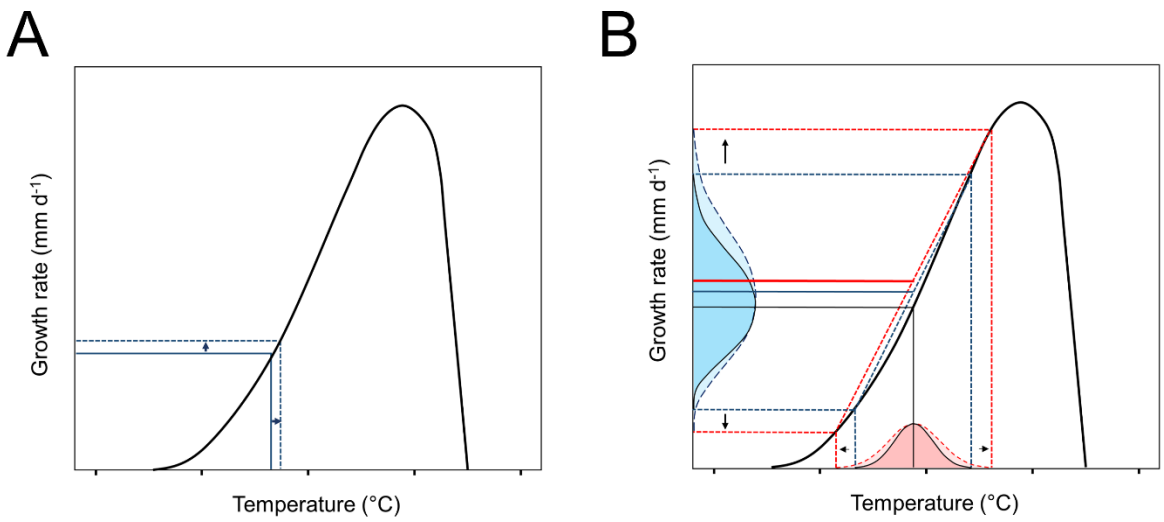

Figure 3. Methods used to estimate chance in growth rate with thermal performance curve (see methods). **A.** Response of growth rate to average temperature (G_{mean}); **B.** Response of growth rate to fluctuation of temperature (G_{mid}).

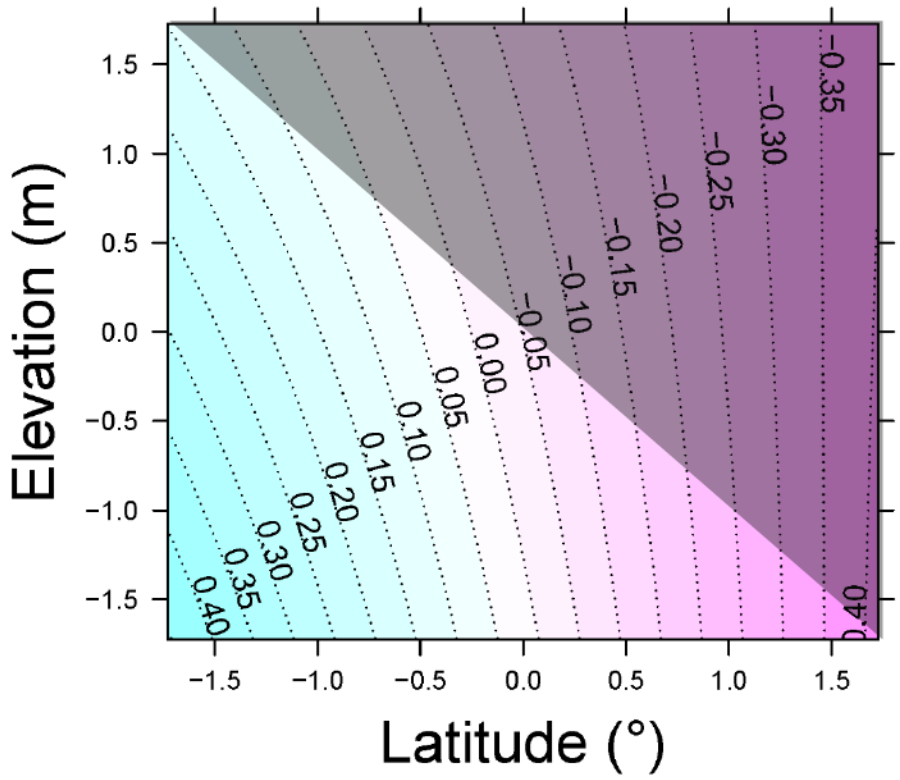

Figure 4. Phenological cline (season duration) of odonates across latitude and elevation in northcentral Europe. Values on isoclines are standardized duration of the flight season. Note: the upper right of the plot (triangle) was shaded because it is not supported by data (see Fig. 2B).

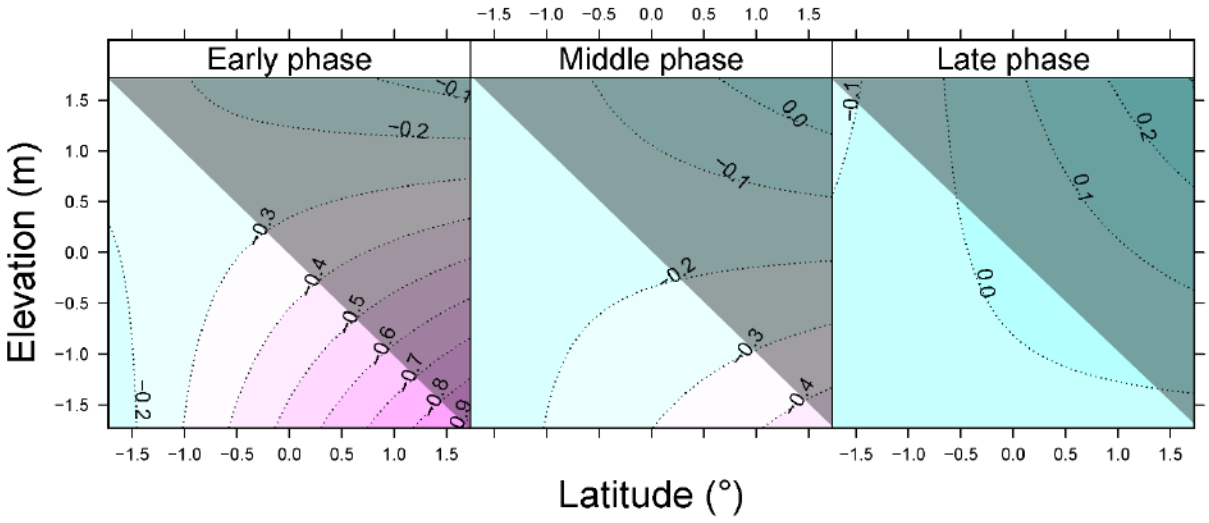

Figure 5. Rates of change in the three phenological phases of odonates across latitude and elevation in northcentral Europe. Early, middle and late phase are quantile 15, 50 and 85. The change in phenology was modelled here as the difference in phenology between 1980-1989 and 2004-2013. Values on isoclines are rate of changes of standardized dates of the year. Note: the upper right of the plot (triangle) was shaded because it is not supported by data (see Fig. 2B).

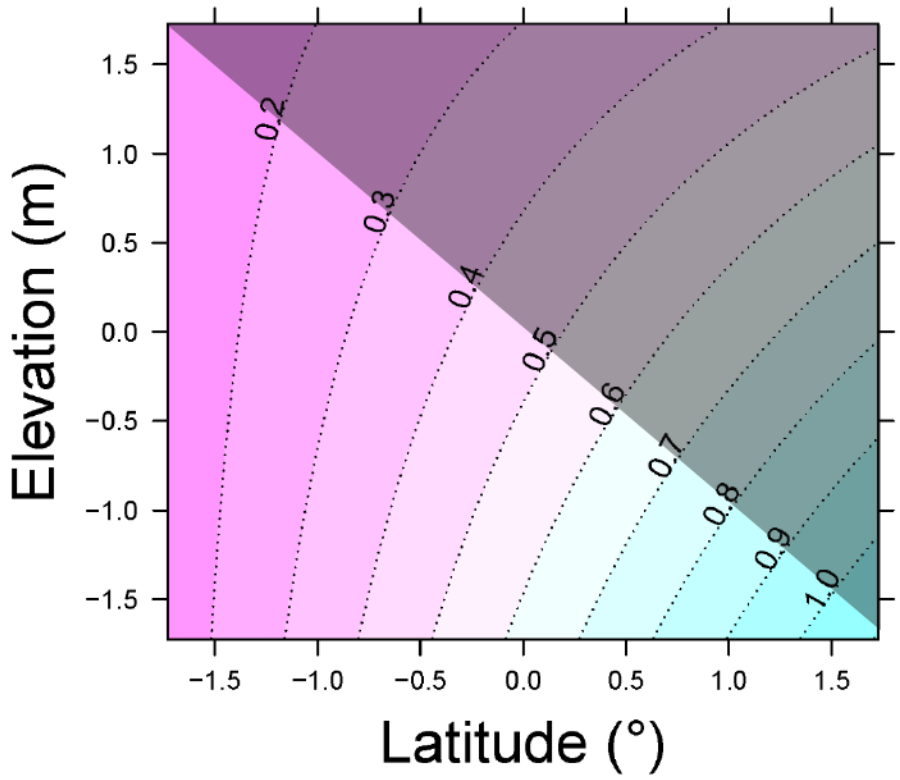

Figure 6. Rate of change in the duration of the flight season of odonates across latitude and elevation in northcentral Europe during 1980-2013. Duration of the season was calculated as the time between quantile 15 and quantile 85 (70% of the season) between 1980-1989 and 2004-2013. Values on isoclines are rate of changes of standardized duration of the season. Note: the upper right of the plot (triangle) was shaded because it is not supported by data (see Fig. 2B).

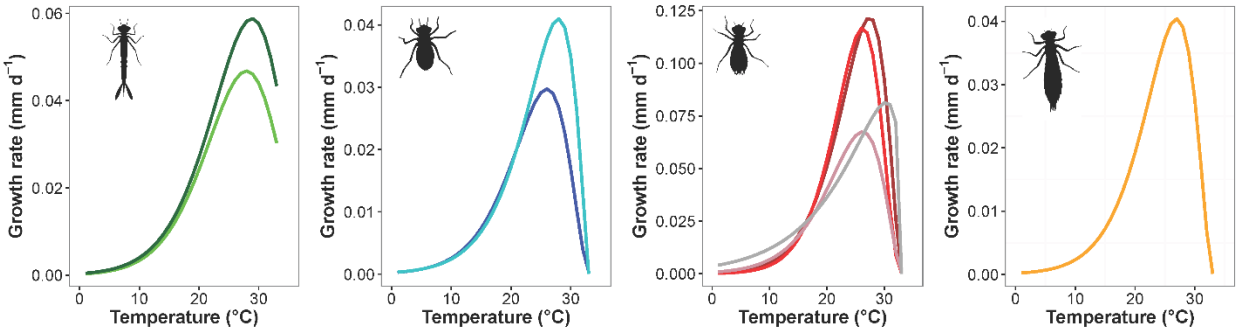

Figure 7. Thermal performance curves of nine species of odonates was estimated in the laboratory. Species were clustered by family and displayed in separate plots. From left to right, families are Coenagrionidae -- *Enallagma cyathigerum* (light green) and *Ischnura elegans* (dark green), Corduliidae -- *Somatochlora metallica* (cyan) and *S. flavomaculata* (lightblue), Libellulidae – *Sympetrum flaveolum* (brown), *S. vulgatum* (red) and *S. danae* (pink), *S. sanguinum* (grey) and Aeshnidae -- *Aeshna juncea* (orange). The curves were fit using the O'Neill function (See Methods). All species showed similar pattern of growth rate across temperature, with a relatively slow increase from the critical minimum to the optimal temperature then a rapid decrease until the critical maximum temperature. However, species varied in the magnitude of growth rate and thermal amplitude. For example, *E. cyathigerum* and *I.elegans* can grow at 33 °C whereas all the other species die at this temperature. Moreover, some species like *S. striolatum* are fast-growing while others like *S. flavomaculata* are slow-growing.

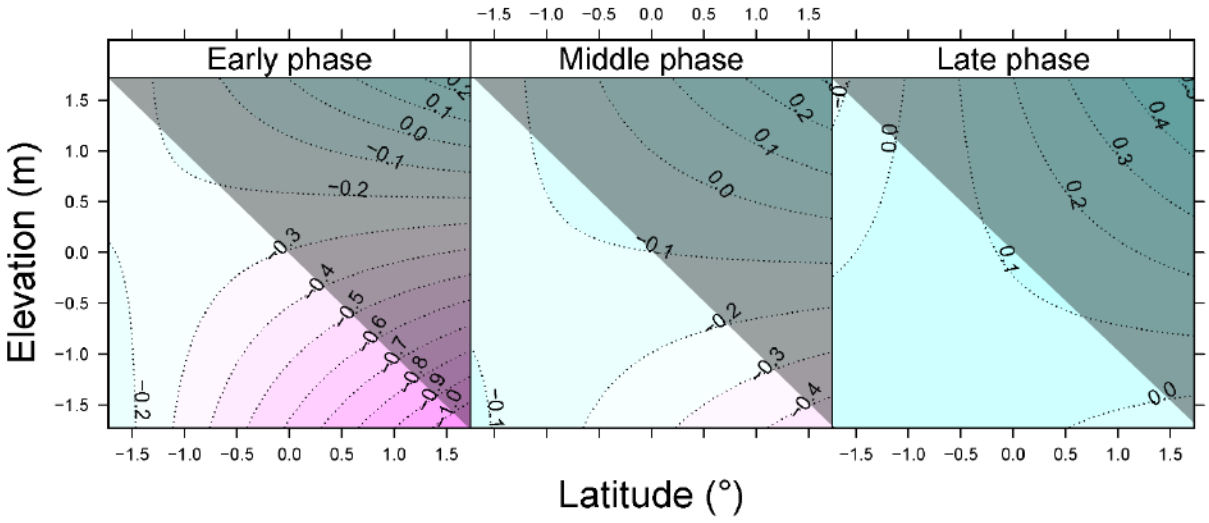

Figure 8. Temporal pattern of three phenological phases of nine species of odonates across latitude and elevation in northcentral Europe between 1980-1989 and 2004-2013. Early, middle and late phase are quantile 15, 50 and 85%. Values on isoclines are rate of changes of standardized dates of the year. Note: the upper right of the plot (triangle) was shaded because it is not supported by data (see Fig. 2B).

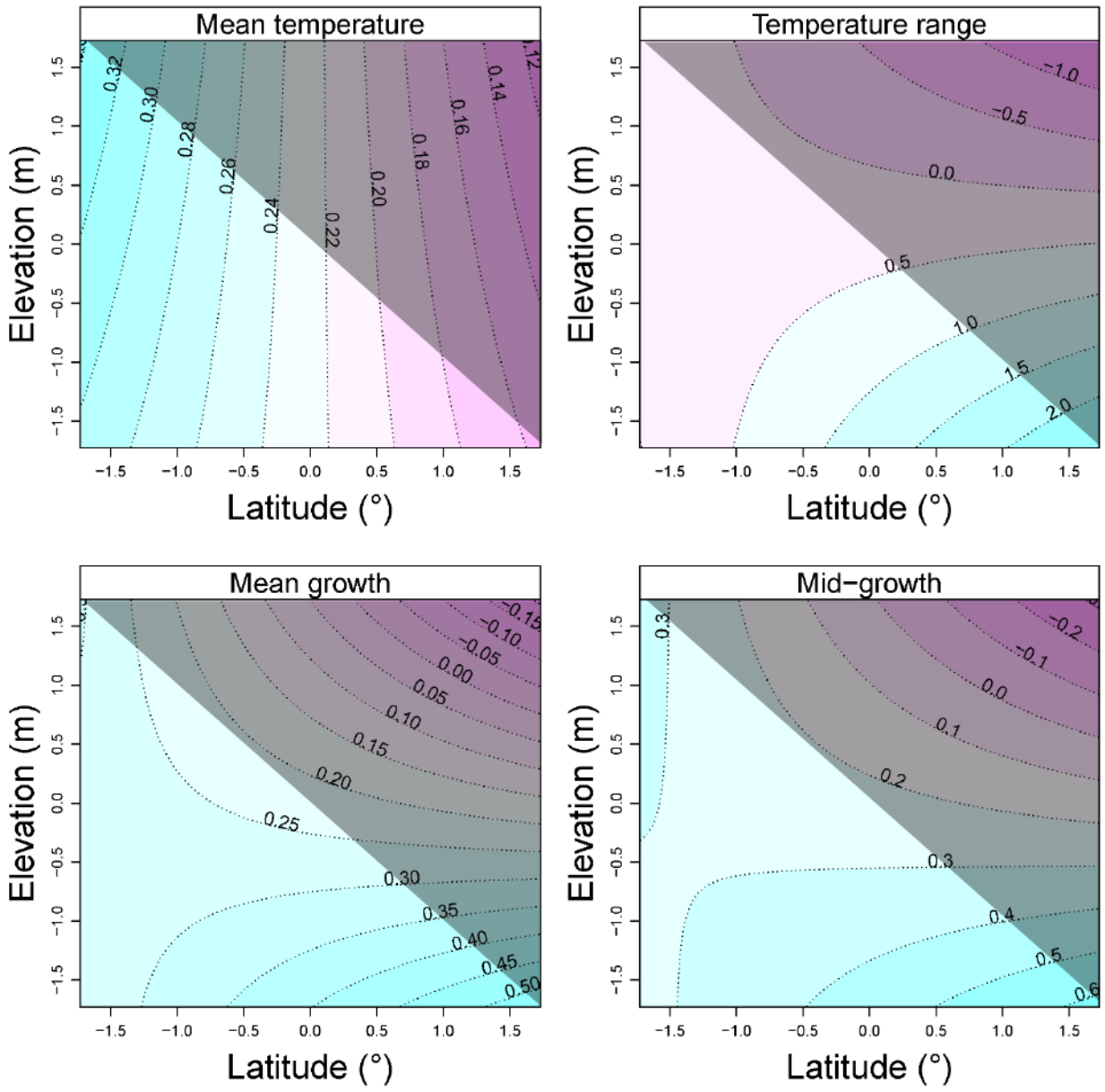

Figure 9. Temporal pattern of the average temperature, range of temperature, average growth and mid-point growth rate of nine species of odonates during the best time window across latitude and elevation in northcentral Europe between 1980-1989 and 2004-2013. Values on isoclines are rate of changes of standardized parameter values. Note: the upper right of the plot (triangle) was shaded because it is not supported by data (see Fig. 2B).

Table 1. Summary statistics of the linear mixed effects model assessing the geographic pattern of the early, middle and late phase of phenology of odonates. A, B and C are early, middle and late phase, respectively.

A	**Random effects**					
	Groups	Name	Variance	Std.Dev.		
	Grid	(Intercept)	81.22	9.012		
	Species	(Intercept)	539	23.216		
	Residual		396.09	19.902		
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	185.7635	3.2838	63	56.569	< 2e-16
	zLat	3.5218	0.5463	205	6.447	8.06E-10
	zAlt	2.3857	0.2997	14739	7.961	1.78E-15
	zLat:zAlt	-0.892	0.1686	13577	-5.292	1.23E-07
B	**Random effects**					
	Groups	Name	Variance	Std.Dev.		
	Grid	(Intercept)	30.98	5.566		
	Species	(Intercept)	625.75	25.015		
	Residual		347.72	18.647		
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	195.1655	3.4525	57	56.53	< 2e-16
	zLat	1.0611	0.3731	181	2.844	0.004973
	zAlt	2.1174	0.2694	6388	7.859	4.44E-15
	zLat:zAlt	-0.4997	0.1513	6245	-3.302	0.000966
C	**Random effects**					
	Groups	Name	Variance	Std.Dev.		
	Grid	(Intercept)	63.75	7.984		
	Species	(Intercept)	699.77	26.453		
	Residual		427.98	20.688		
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	205.6407	3.6887	59	55.749	< 2e-16
	zLat	-1.2919	0.5029	195	-2.569	0.0109
	zAlt	1.4892	0.3073	10727	4.846	1.27E-06
	zLat:zAlt	-0.1539	0.1727	10037	-0.891	0.3728

Table 2. Summary statistics of the linear mixed effects model assessing the geographic pattern of season duration of odonates.

Random effects					
Groups	Name	Variance	Std.Dev.		
Site	(Intercept)	147.8	12.16		
Species	(Intercept)	140.8	11.87		
Residual		8888.3	94.28		
Fixed effects					
	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	32.0450	2.0308	115	15.779	< 2e-16
zLat	-5.1782	0.7993	236	-6.478	5.34e-10
zAlt	-1.0388	0.5108	15788	-2.034	0.0420
zLat:zAlt	0.6962	0.2829	16318	2.461	0.0139

Table 3. Summary statistics of the linear mixed effects model assessing the temporal pattern of the duration of phenology of odonates.

Random effects					
Groups	Name	Variance	Std.Dev.		
Grid	(Intercept)	159.5	12.63		
Species	(Intercept)	113.5	10.65		
Residual		409.5	20.24		
Fixed effects					
	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	17.4806	1.8965	128	9.218	8.88e-16
zyear	6.5481	0.1046	52167	62.590	< 2e-16
zLat	-4.6112	0.7065	233	-6.527	4.15e-10
zAlt	-0.7315	0.3117	25209	-2.347	0.01896
zyear:zLat	0.2980	0.1130	52051	2.638	0.00835
zyear:zAlt	-1.8925	0.1680	50789	-11.267	< 2e-16
zLat:zAlt	0.7505	0.1754	23187	4.279	1.88e-05
zyear:zLat:zAlt	-0.7007	0.1070	50261	-6.547	5.92e-11

Table 4. Summary statistics of the linear mixed effects model assessing the temporal pattern of the early, middle and late phase of phenology of odonates. A, B and C are early, middle and late phase, respectively.

A	**Random effects**					
	Groups	Name	Variance	Std.Dev.		
	Grid	(Intercept)	49.33	7.024		
	Species	(Intercept)	658.02	25.652		
	Residual		5903.82	76.836		
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	1.807e+02	3.569e+00	5.800e+01	50.622	< 2e-16
	zyear	-3.594e+00	9.032e-02	5.201e+04	-39.792	< 2e-16
	zLat	6.727e+00	5.300e-01	2.010e+02	12.692	< 2e-16
	zAlt	4.976e+00	4.067e-01	8.415e+03	12.237	< 2e-16
	zyear:zLat	-1.834e+00	1.342e-01	4.674e+04	-13.668	< 2e-16
	zyear:zAlt	1.185e+00	1.765e-01	5.133e+04	6.711	1.95e-11
	zLat:zAlt	6.511e-01	2.277e-01	1.000e+04	2.860	0.00425
	zyear:zLat:zAlt	1.143e+00	1.254e-01	4.993e+04	9.114	< 2e-16

B	**Random effects**					
	Groups	Name		Variance	Std.Dev.	
	Grid	(Intercept)		23.36	4.834	
	Species	(Intercept)		825.03	28.723	
	Residual			6057.25	77.828	
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	1.955e+02	3.946e+00	5.500e+01	49.548	< 2e-16
	zyear	-2.010e+00	9.131e-02	5.166e+04	-22.009	< 2e-16
	zLat	2.756e+00	4.186e-01	1.760e+02	6.583	5.09e-10
	zAlt	4.797e+00	3.937e-01	3.261e+03	12.186	< 2e-16
	zyear:zLat	-1.532e-01	1.349e-01	3.934e+04	-1.136	0.256
	zyear:zAlt	1.241e+00	1.783e-01	5.044e+04	6.960	3.44e-12
	zLat:zAlt	1.142e+00	2.215e-01	4.251e+03	5.155	2.65e-07
	zyear:zLat:zAlt	7.358e-01	1.264e-01	4.735e+04	5.820	5.92e-09

C	**Random effects**					
	Groups	Name	Variance	Std.Dev.		
	Grid	(Intercept)	53.06	7.284		
	Species	(Intercept)	952.67	30.865		
	Residual		7551.79	86.901		
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	211.7408	4.2718	57	49.567	< 2e-16
	zyear	0.4833	0.1021	51935	4.733	2.22e-06
	zLat	-0.1888	0.5668	172	-0.333	0.739
	zAlt	4.2934	0.4560	6213	9.414	< 2e-16
	zyear:zLat	0.6953	0.1515	44647	4.588	4.48e-06
	zyear:zAlt	1.0257	0.1995	51059	5.140	2.75e-07
	zLat:zAlt	1.7000	0.2555	7567	6.653	3.08e-11
	zyear:zLat:zAlt	0.7604	0.1417	49168	5.366	8.07e-08

Table 5. Summary statistics of the linear mixed effects model assessing the temporal pattern of average temperature of the best time window that explain the phenological changes of odonates.

Random effects					
Groups	Name	Variance	Std.Dev.		
Grid	(Intercept)	0.6125	0.7826		
Residual		1.0304	1.0151		
Fixed effects					
	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	13.86983	0.06557	1530	211.533	< 2e-16
z.year	0.35480	0.01425	50490	24.891	< 2e-16
zLat	-0.44075	0.07037	1530	-6.263	3.64e-09
zAlt	-0.24281	0.07635	1530	-3.180	0.00178
z.year:zLat	-0.05160	0.01530	50490	-3.373	0.00075
z.year:zAlt	0.01371	0.01660	50490	0.826	0.40878
zLat:zAlt	0.04254	0.04769	1530	0.892	0.37376
z.year:zLat:zAlt	-0.02001	0.01037	50490	-1.930	0.05369

Table 6. Summary statistics of the linear mixed effects model assessing the temporal pattern of temperature range of the best time window that explain the phenological changes of the early phase of odonates.

Random effects					
Groups	Name	Variance	Std.Dev.		
Grid	(Intercept)	0.724767	0.85133		
Species	(Intercept)	0.007351	0.08574		
Residual		26.517149	5.14948		
Fixed effects					
	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	8.56016	0.08625	97.00	99.245	< 2e-16
zyear	0.05453	0.01446	8843.00	3.772	0.000163
zLat	0.36748	0.07701	177.00	4.772	3.8e-06
zAlt	-0.19398	0.06674	2938.00	-2.906	0.003683
zyear:zLat	0.04161	0.02207	8783.00	1.885	0.059448
zyear:zAlt	-0.05329	0.02938	8838.00	-1.814	0.069717
zLat:zAlt	-0.05161	0.03756	3932.00	-1.374	0.169507
zyear:zLat:zAlt	-0.05734	0.02000	8814.00	-2.867	0.004148

1.6. Supplementary information

Table S1. List of species included in the analysis of long-term changes in phenology. Total number of species is 54.

Anisoptera		**Zygoptera**	
Species	Family	Species	Family
Aeshna cyanea	Aeshnidae	*Calopteryx splendens*	Calopterygidae
Aeshna grandis	Aeshnidae	*Calopteryx virgo*	Calopterygidae
Aeshna isoceles	Aeshnidae	*Ceriagrion tenellum*	Coenagrionidae
Aeshna juncea	Aeshnidae	*Coenagrion hastulatum*	Coenagrionidae
Aeshna mixta	Aeshnidae	*Coenagrion lunulatum*	Coenagrionidae
Aeshna viridis	Aeshnidae	*Coenagrion mercuriale*	Coenagrionidae
Anax imperator	Aeshnidae	*Coenagrion puella*	Coenagrionidae
Anax parthenope	Aeshnidae	*Coenagrion pulchellum*	Coenagrionidae
Brachytron pratense	Aeshnidae	*Enallagma cyathigerum*	Coenagrionidae
Cordulegaster boltonii	Cordulegastridae	*Erythromma lindenii*	Coenagrionidae
Somatochlora flavomaculata	Corduliidae	*Erythromma najas*	Coenagrionidae
Somatochlora metallica	Corduliidae	*Erythromma viridulum*	Coenagrionidae
Cordulia aenea	Cordulinae	*Ischnura elegans*	Coenagrionidae
Gomphus pulchellus	Gomphidae	*Ischnura pumilio*	Coenagrionidae
Gomphus vulgatissimus	Gomphidae	*Pyrrhosoma nymphula*	Coenagrionidae
Crocothemis erythraea	Libellulidae	*Lestes barbarus*	Lestidae
Leucorrhinia dubia	Libellulidae	*Lestes dryas*	Lestidae
Leucorrhinia pectoralis	Libellulidae	*Lestes sponsa*	Lestidae
Leucorrhinia rubicunda	Libellulidae	*Lestes virens*	Lestidae
Libellula depressa	Libellulidae	*Lestes viridis*	Lestidae
Libellula fulva	Libellulidae	*Platycnemis pennipes*	Platycnemididae
Libellula quadrimaculata	Libellulidae		
Orthetrum brunneum	Libellulidae		
Orthetrum cancellatum	Libellulidae		
Orthetrum coerulescens	Libellulidae		
Sympetrum danae	Libellulidae		
Sympetrum depressiusculum	Libellulidae		
Sympetrum flaveolum	Libellulidae		
Sympetrum fonscolombii	Libellulidae		
Sympetrum pedemontanum	Libellulidae		
Sympetrum sanguineum	Libellulidae		
Sympetrum striolatum	Libellulidae		
Sympetrum vulgatum	Libellulidae		

Table S2. List of species and populations used in the laboratory experiments measuring thermal performance curves.

Order	Family	Species	No. females	Sampling location	Elevation (m)
Zygoptera	Coenagrionidae	*Ischnura elegans*	5	47°23'49.36"N 8°32'36.03"E	481
			5	47°18'15.19"N 8°47'53.87"E	539
			5	47°11'13.81"N 9°19'52.72"E	1162
			5	46°57'55.25"N 6°34'28.01"E	1039
	Coenagrionidae	*Enallagma cyathigerum*	5	47° 3'56.27"N 9°15'17.84"E	1902
			5	46°56'35.29"N 9° 5'54.06"E	1968
			5	47°23'49.36"N 8°32'36.03"E	481
			5	47°18'15.19"N 8°47'53.87"E	539
Anisoptera	Corduliidae	*Somatochlora alpestris*	5	47° 3'56.27"N 9°15'17.84"E	1902
			4	46°55'50.73"N 9° 4'28.73"E	1995
	Corduliidae	*Somatochlora flavomaculata*	2	47°18'15.19"N 8°47'53.87"E	539
			1	47°25'37.20"N 8°29'14.81"E	441
	Corduliidae	*Somatochlora metallica*	2	46°32'4.36"N 9°38'34.10"E	1964
			1	46°47'4.43"N 9°40'59.07"E	1738
	Libellulidae	*Sympetrum vulgatum*	5	47°18'15.19"N 8°47'53.87"E	539
			5	47°23'49.36"N 8°32'36.03"E	481
	Libellulidae	*Sympetrum striolatum*	5	47°23'49.36"N 8°32'36.03"E	481
			5	47°18'15.19"N 8°47'53.87"E	539
	Libellulidae	*Sympetrum danae*	5	46°44'8.35"N 9°33'25.73"E	1514
			5	46°44'22.52"N 9°33'18.78"E	1485
	Libellulidae	*Sympetrum sanguineum*	3	47°18'15.19"N 8°47'53.87"E	539
	Aeshnidae	*Aeshna juncea*	5	46°47'58.26"N 9°40'40.45"E	1988
			5	46°44'8.35"N 9°33'25.73"E	1514

Table S3. Estimation of the slope of temporal pattern of average temperature of all months relevant to development of odonates.

Window	Slope	SE	df	95%LCL	95%UCL
August	0.0209	0.0026	26775	0.0157	0.0260
October	0.0225	0.0026	26775	0.0174	0.0276
November	0.0297	0.0026	26775	0.0246	0.0348
March	0.0297	0.0025	26775	0.0248	0.0345
May	0.0297	0.0025	26775	0.0248	0.0346
July	0.0303	0.0025	26775	0.0254	0.0351
June	0.0316	0.0025	26775	0.0267	0.0365
September	0.0330	0.0026	26775	0.0278	0.0381
April	0.0593	0.0025	26775	0.0545	0.0642

Table S4. Slope of the mixed effects model assessing sensitivity of phenology (three phases) to temperature for each time window (month). Values in bold are the steepest slopes.

Window	estimate	std.error	statistic	p.value	Quantile (%)
October	-0.619	0.127	-4.864	<0.0001	15
November	0.178	0.116	1.530	0.126	15
March	-0.700	0.117	-5.960	<0.0001	15
April	-4.029	0.147	-27.326	<0.0001	15
May	**-6.409**	**0.167**	**-38.275**	<0.0001	**15**
June	**-6.670**	**0.173**	**-38.464**	<0.0001	**15**
July	-2.605	0.143	-18.211	<0.0001	15
August	-3.381	0.151	-22.364	<0.0001	15
September	-1.940	0.154	-12.595	<0.0001	15
October	0.175	0.126	1.391	0.164	50
November	0.172	0.115	1.491	0.136	50
March	-0.388	0.117	-3.328	0.001	50
April	-2.257	0.147	-15.346	<0.0001	50
May	**-3.328**	**0.168**	**-19.854**	<0.0001	**50**
June	**-3.269**	**0.173**	**-18.883**	<0.0001	**50**
July	-0.709	0.142	-4.999	<0.0001	50
August	-0.787	0.150	-5.259	<0.0001	50
September	-0.102	0.152	-0.669	0.504	50
October	1.271	0.134	9.460	<0.0001	85
November	0.698	0.123	5.661	<0.0001	85
March	0.011	0.125	0.086	0.931	85
April	-0.096	0.158	-0.609	0.543	85
May	0.149	0.180	0.826	0.409	85
June	0.674	0.185	3.637	<0.0001	85
July	1.347	0.151	8.914	<0.0001	85
August	**2.176**	**0.159**	**13.667**	<0.0001	**85**
September	**2.028**	**0.162**	**12.495**	<0.0001	**85**

Impact of climate change on insect phenology declines with latitude but not with elevation

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In many organisms, the timing of life-cycle events – known as phenology – has become substantially earlier in recent decades as a result of climate warming. These changes can have important ecological, economic and health consequences. Here we compile long-term observations of 54 species of adult Odonata in Europe to show that sensitivity of apparent phenology (S_T) to temperature changed from 1980 to 2013 along latitude and elevation. We found an overall decline of S_T, that is, the phenology of odonates became less sensitive to temperature in recent years. However, the change in S_T varied with latitude and elevation. S_T declined at both low and high latitudes, albeit more so at the latter, but it did not change at high elevation. Based on our results, photoperiod limitation and loss of chilling were the most likely factors underlying the spatiotemporal pattern of S_T. We suggest that sensitivity to temperature of ectotherms may either increase or decrease in the future depending primarily on the warming of winter and the current timing of the life-history trait, that is whether the phenological event is before or after the peak photoperiod of the year.

Keywords: Sensitivity to temperature, odonates, dragonflies, warming, photoperiod, winter warming, chilling

Climate warming in recent decades has substantially altered the phenology of many animals and plants (Parmesan 2006). In most cases, the mechanism underlying the response appears to be beneficial phenotypic plasticity: individual organisms adjust the timing of their activity and development to track changes in local temperature (Charmantier et al. 2008, Gienapp et al. 2008, Phillimore et al. 2010). However, it remains unknown whether plasticity can continue to match temperature changes in the future. It is possible that plasticity will fail to keep pace, or will even decline, as organisms encounter novel environmental conditions and keep approaching the limits of phenotypic expression (DeWitt 1998, Ghalambor et al. 2007). This question is critically important because other responses to climate change – microevolutionary adaptation and shifts in the geographic range – may be encumbered by constraints (Davis et al. 2005, Chevin et al. 2010, Hoffmann and Sgrò 2011, Bateman et al. 2013). We addressed this issue by estimating changes in temperature-induced plasticity in European members of the insect order Odonata (dragonflies and damselflies) from 1980 to 2013, a period during which temperatures in Europe increased substantially (Intergovernmental Panel on Climate Change 2014).

Predicting long-term warming effects on the phenology of ectotherms in the temperate zone is challenging. A phenological shift may move the sensitivity of a species relative to the annual fluctuations in mean temperature and daily temperature differences, or to the environmental photoperiod (Körner and Basler 2010), which itself may interact with warming, inducing either a positive or a negative physiological response to temperature (Bradshaw and Holzapfel 1977, Corbet 1980, Ward and Stanford 1982, Bale et al. 2002). Moreover, there is a potential carry-over effect of winter warming on metabolic rates in later life stages (Stålhandske et al. 2014, Stålhandske et al. 2017), which may alter the expected thermal response of species to warming.

So far, there has been little progress in understanding the effects of temperature fluctuations, winter warming and the photoperiod on the magnitude of the phenological response to warming in ectotherms. The current study focused on the early phase of phenology, defined as the date during which 15% of all observations per season were registered. This phase reflects the start of the flight season and thus indicates how the population responds to climate warming. Moreover, this phenological phase showed a significant advancement during 1980-2013 (see Chapter I). Here, long-term observation data on 54 European species of adult odonates (dragonflies and damselflies) are analyzed to assess the potential factors that explain the spatiotemporal pattern of thermal sensitivity of the early phase of phenology to warming.

To assess potential temporal changes in adaptive plasticity, we first determined a general (across all species) and species-specific thermal sensitive period (TSP), defined as the seasonal time window during which average temperature best explained year-to-year variation in phenology. Then, we estimated S_T – the number of days the early phase shifts per 1°C warming – as the slope between Julian date (day of the year) and average temperature of the TSP in a linear mixed effects (LME) model with species entered as a random effect (see Methods…). The temporal pattern of S_T was first calculated with a LME model including average temperature-by-year interaction and species, site and TSP as random effects. While year and temperature had a negative effect on the early phase, temperature-by-year interaction had a significant positive effect (Table S1), revealing a decline of S_T to TSP during 1980-2013. We then divided the 34 years into two equal 17-year periods (1980-1996 and1997-2013) and calculated S_T in each period. There was a 11.4% decline in S_T across all potential TSP, with S_T changing from -0.59 $d \cdot °C^{-1}$ [95%CI: -0.63 to -0.55] in the period of 1980-1996 to -0.52 $d \cdot °C^{-1}$ [-0.55 to -0.50] between 1997 and 2013. Even with a species-specific TSP, S_T declined by 35.3% overall, from -2.56 $d \cdot °C^{-1}$ [-2.94 – -2.18] in 1980-1996 to -1.65 $d \cdot °C^{-1}$ [-1.99 – -1.31] in 1997-2013. Combined, these results suggest that the phenology of odonates is currently not as sensitive as it used to be a few decades ago.

To investigate the geographic variation of S_T and potential spatiotemporal changes, we divided the study area into latitudinal and elevational districts using a thermal equivalence approach (Pickett 1989) (hereafter referred to as low district and high latitude and high elevation; Fig. 1A, Supplementary information). S_T was then calculated for each district. Overall, S_T was similar at low and high latitude with -0.17 $d \cdot °C^{-1}$ [95%CI: -0.19 – -0.15] and -0.16 $d \cdot °C^{-1}$ [-0.18 – -0.16], respectively, but S_T was lower at high elevation with -0.04 $d \cdot °C^{-1}$ [-0.09 – 0.005]. Although there was a general decline of S_T across the study area, the temporal change was significant at low district and high latitude, but not at high elevation (Fig. 1B, Table 1). The magnitude of the temporal decline in S_T was greater at high latitude (56%: from -0.36 $d \cdot °C^{-1}$ to -0.16 $d \cdot °C^{-1}$) than at low district (26.2%: from -0.26 $d \cdot °C^{-1}$ to -0.19 $d \cdot °C^{-1}$). These findings reveal differences in the response of odonate phenology to warming if it occurs along latitudes or elevations, respectively.

We evaluated three non-mutually exclusive explanations for the long-term dynamics of thermal sensitivity of phenology across latitude and elevation: (1) due to the non-linear relationship between temperature and development (thermal performance curve), the magnitude of the thermal reaction norm depends on ambient temperature (T_a), with increasing temperature changing S_T

positively if T_a is on the left hand side of the optimal temperature and negatively if T_a is on the right hand side of the optimal temperature; (2) the shift in phenology influences S_T by exposing final-instar larvae to shorter photoperiods; and (3) exposure to warmer winters in recent years has affected the response of larvae to temperature in the spring. In the following, we evaluate to what extent currently available data support any of these explanations.

Our first hypothesis proposes that historical variation in thermal regimes changed thermal sensitivity of phenology. Both average and diurnal fluctuation of temperature has increased significantly in all districts (Fig. 2A, B), but the long-term increases were greater at high latitude than at high elevation. Due to the non-linear thermal performance curve (TPC) of ectotherms (Deutsch et al. 2008), the thermal shift due to warming could alter S_T. Depending on the position of ambient temperature in the TPC, the developmental response to a given increase in temperature may either increase or decrease (Fig. 2C). To determine how warming has affected thermal sensitivity phenology of odonates, we estimated the TPC of nine species from different families (see Chapter I) and simulated the thermal response of their growth rate to the historical change in temperature of the TSP during 1980-1996 and 1997-2013, respectively. The changes in thermal regimes translated into a predicted increase in the thermal sensitivity of growth rate within all geographic districts (Fig. 2D), which contrasts with the observation that S_T has mostly declined across the study area (Fig. 1B).

The second hypothesis proposes that changes in S_T are constrained by differences in the photoperiod to the extent that the phenology is advanced. Photoperiod is of general importance in regulating the timing of insect life cycles (Tauber et al. 1986), and is a better cue for the emergence of adult odonates than temperature that varies between years. Odonates emerge later at high than at low latitudes and elevations (Fig. 3A), and this results in geographic variation in the photoperiod at the time of emergence. This implies that the phenological advancement recorded in odonates would lead to either a photoperiod limitation or augmentation depending on whether the phenological event occurs before or after the summer solstice (Fig. 3B). At low and high latitude, the early phase of phenology occurs at about the summer solstice, whereas at high elevation it occurs after the solstice (Fig. 4A). As a consequence, the phenological shift occurring between 1980 and 2013 shortened the photoperiod in the low and high-latitude districts, but not in the high-elevation district (Fig. 4B). Although the geographic pattern in odonate emergence relative to the photoperiod corresponds to that of the changes in S_T, its implication in buffering warming effects

on phenology remains unclear due to the relatively weak decline in the photoperiod during the early phase (15.1 min at low and 22.0 min at high latitude) (Table 2a). While the changes in day length may be modest in the early phase, they might be stronger at the larval stage (prior to adult emergence), when individuals use photoperiodic cues to assess the time for development and emergence (Nylin and Gotthard 1998). To test this prediction, we calculated the temporal pattern of the photoperiod at 30 days before the early phase, when the larvae that are expected to emerge in the same year are in their last larval instars. We found a greater reduction of the photoperiod than during the early adult phase (Fig. 4B): 37.0 min for the low district, 51.7 min for high latitude, and 9.6 min for high elevation (Table 2b). Therefore, a shortened photoperiod may, to some extent, have contributed to the decline in S_T of the early phase.

The third hypothesis states that exposure to periods of winter cold – known as "chilling" (Bale and Hayward 2010) – has declined over time, and this affects the response to temperature variation in the spring. Deep winter chilling is known to increase the development rate during the following spring in some insects (Bale and Hayward 2010, Williams et al. 2015). Under this hypothesis, the sensitivity to temperature also increases following colder winters because a higher maximum development rate offers greater scope for plasticity. To test this hypothesis, we first examined whether the number of cold winter days declined over time in our study area, by regressing the number of days below a chilling threshold of 6°C (Koch 2015) against year. The number of chilling days declined significantly at low and high latitudes (Fig. 5A, Table 3), but did not change at high elevation. The magnitude of the decline was highest at high latitude. This geographic variation in the temporal changes of chilling matches the geographic pattern of changes in S_T (Fig. 1B). Next, we calculated the relationship between the post-winter cumulative temperature required to complete development (between 1 March and the date of the early phase) and the number of chilling days during the preceding winter. We found a significant negative relationship in all districts (Fig. 5B, Table 4), suggesting that odonate larvae required less temperature to emerge in years with more chilling days, which may explain why S_T declined in regions where winters became warmer and shorter.

In conclusion, this study investigated three hypotheses to explain the spatiotemporal pattern of thermal sensitivity of phenology of odonates and revealed the potential implication of two hypotheses. First, photoperiod has decreased substantially for the larval stage during the last three decades, which might have reduced the S_T of phenology. Second, winters have become

warmer such that the thermal response of phenology to spring and summer temperature declined. Further studies need to experimentally test the interaction between photoperiod limitation and chilling loss in affecting S_T.

Understanding the factors affecting the intensity of shifts in phenology across major geographic gradients is critical to predicting future seasonal patterns of biodiversity. A decline in S_T of 40% has been observed for seven European tree species, mostly due to winter warming (Fu et al. 2015). These results are similar to our findings, which suggests that the decline in S_T might be widespread across animals and plants. Our study further shows that the changes in S_T vary along geographic gradients (latitude and elevation), most likely because of differential temporal winter warming and photoperiodic changes. Due to the similar thermal adaptation of ectotherms (the highest diversified group of organisms on earth), it is likely that the phenology of a large proportion of biodiversity will respond to temperature equally or more than expected at higher elevations (≈ 600-1200 m) but less than expected at higher latitudes (≈52-58° N). The microevolutionary implications of these differential thermal responses and their consequences for biotic interactions and global biodiversity are the next research challenges.

2.1. Methods

2.1.1. Dataset

Our dataset of odonate observations across northcentral Europe (latitude from 46 °N to 69 °N and elevation from -5 m to 2750 m) was assembled for the period of 1980 – 2013. Data were obtained for Switzerland (Centre Suisse de Cartographie de la Faune), Germany (Arbeitskreis zum Schutz und zur Kartierung der Libellen in Nordrhein-Westfalen), Belgium (Instituut voor Natuuren Bosonderzoek), the Netherlands (EIS Kenniscentrum Insecten en andere ongewervelden), the United Kingdom (British Dragonfly Society), and Sweden (the website http://svalan.artdata.slu.se/bugs/). The complete dataset consisted of ~2.53 million observations of adult individuals from 78 odonate species. A species was included if there were ≥ 8 observations per year in ≥ 8 years extending over a range of ≥ 12 years for each of the periods 1980-1996 and 1997-2013. This data truncation resulted in a total of 54 species.

We obtained monthly mean and daily minimum/maximum temperatures from weather stations in Switzerland (63 stations from http://www.meteosuisse.admin.ch/), Germany (41

stations from http://www.dwd.de/), the Netherlands (1 station from http://www.knmi.nl/home), Belgium (3 stations from http://www.meteo.be/), the United Kingdom (21 stations from http://www.metoffice.gov.uk/) and Sweden (29 stations from http://luftwebb.smhi.se/). Although we used air temperature instead of water temperature because of data availability, the latter have been shown to be correlated (Piccolroaz et al. 2013).

2.1.2. Division of geographic gradients

The study area was divided into three districts (low district, high latitude and high elevation) based on a latitude-for-elevation temperature lapse rate of 94.3 m in elevation for each 1° in latitude (see Fig. 1A). Each district spans 6° in latitude and ~566 m in elevation. High-latitude and high-elevation districts have similar annual temperature. This lapse was determined based on air temperature of weather stations, and is comparable with reports in the literature (Jacobson 2005).

2.1.3. Time window selection

We calculated the15th percentile (early phase) of phenology by Julian date for each combination of year, species and district. We determined the most sensitive time window (TSP) that best explains the phenological changes of (1) each species (species-specific window) with a linear regression of the phenological phase by mean temperature of a TSP spanning from one to five months (March-July), and (2) of all species (general window) with a LME model including species as a random effect. The best TSP was selected based on the lowest AIC.

2.1.4. Analyses

All statistical analyses were performed in R version 3.2.2 (R Development Core Team 2017), using the *lme4* package (Bates et al. 2015) for linear mixed effect models (LME). Sensitivity to temperature (S_T) was calculated as the slope of the regression between the early phenological phase (15th percentile) and mean temperature of TSP, and it describes the rate at which apparent phenology shifts per 1 °C warming. To assess the temporal pattern of thermal sensitivity, we used two different models:

S_T-continuous-model (continuous space-time variables):

$$phenology \sim temp \times year + (1|Species) + (1|grid)$$

S_T-discrerte-model (discrerte space-time variables):

$$phenology \sim temp \times district \times period + (1|Species)$$

where *phenology* is the early phase (15th percentile); *temp* is the average temperature of TSP of a given year, *district* is the geographic division of the gradients (low district, high latitude and high

elevation), *period* is a factor with the two levels 1980-1996 and 1997-2013, respectively, grid is 1°latitude × 1°longitude and *(1|Species)* and *(1|grid) are* random effects. S_T was then calculated for each of the two periods and tested for potential differences across districts using the *lstrends* function in the R package *lsmeans* (Lenth 2015). The temporal pattern of S_T was assessed for each of the geographic districts to unravel geographic patterns across latitude and elevation. The latter was conducted for all potential TSP by introducing TSP as a random effect and for selected species-specific TSP.

Since S_T-discrerte-model and S_T-continuous-model showed a similar pattern, we used only S_T-discrerte-model to test the three hypothesis (change in ST of growth rate, photoperiodic changes, and winter warming) because S_T-continuous-model is computationally demanding. To assess the temporal pattern of the average and amplitude of temperature of the best TSP and photoperiod during 1980-2013, LME models regressing the response variable against year were fitted with species as a random effect. To reveal potential temporal changes in the sensitivity of growth rate, we used the thermal performance curve of nine species and simulated the warming of the best TSP that occurred in each geographic district during the two periods 1980-1996 and 1997-2013, respectively. Then, we compared the sensitivity of growth rate to temperature with an LME regressing growth rate against the interaction term of temperature-by-period.

The number of chilling days were as calculated from the first of October to the first of March by counting the number of days in which the average temperature fell below 6°C, a temperature below which there is no development (Koch 2015). We assessed the temporal pattern of chilling days during 1980-2013 with a simple linear model. To confirm the relationship between chilling and thermal requirements during the growth season, we modelled cumulative temperature (≥ 6°C from the first of March to the early phenological phase) during the relevant period for adult emergence for each combination of species, year and district, with an LME including the number of chilling days and district chilling threshold as predictors and species as a random effect.

2.1.5. Thermal performance curves

We estimated thermal performance curves (TPCs) for nine odonate species by rearing larvae individually in 200-ml cups in the laboratory at six temperatures (18, 21, 24, 27, 30, and 33°C). Depending on the species, between 3 and 20 females contributed eggs to the experiment, and we reared two replicate larvae from each female (see Chapter I: Supplementary information). The experiment began immediately after hatching and continued until metamorphosis

(zygopterans) or for 60 days (anisopterans). Larvae were fed ad libitum every day with *Artemia* sp. nauplii. The growth rate was calculated as the change in head width per day. This trait is a good indicator of body size and larval instar (Corbet 1999). Since larvae grow in a discrete manner (body size increases after molting), development rate and growth rate are highly correlated.

Parameters of TPCs were estimated by fitting the O'Neill function (Spain 1982) to the data for each species separately. This model assumes that the development rate increases exponentially with temperature on the left side of the thermal optimum (T_{opt}), peaks at the value k, and then decreases abruptly toward the critical maximum temperature at which development stops (T_{max}):

$$\Phi(T) = k\left(\frac{T_{max}-T}{T_{\max}-T_{opt}}\right)^{p} \exp\left(\frac{p(T-T_{opt})}{T_{max}-T_{opt}}\right) \quad ,$$

where $\Phi(T)$ is the observed development rate at temperature T, k is the maximum development rate, $p = \frac{1}{400} J^2 \left(1 + \sqrt{1 + \frac{40}{J}}\right)^2$, and $J = (Q_{10} - 1)\left(T - T_{opt}\right)$.

We used the growth rate as a measure of development. To assess the changes in growth rates, we calculated the average growth rate for each year using average temperature of the TSP for each of the nine species during the period 1980-2013.

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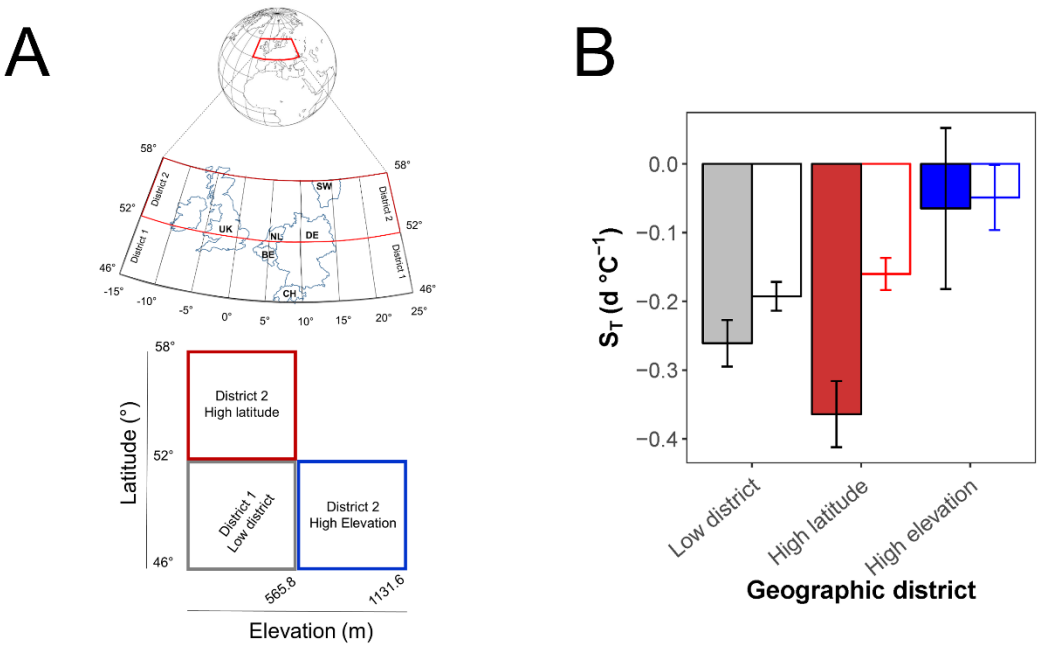

Figure 1. A. The study area was divided into three districts: a low district (common among latitude and elevation) and a high latitudinal and elevational district. The division was made such that high latitudinal and elevational districts have similar average annual temperature. **B.** Changes in S_T of the early phase between 1980-1996 (filled bars) and 1997-2013 (open bars) in the three districts used.

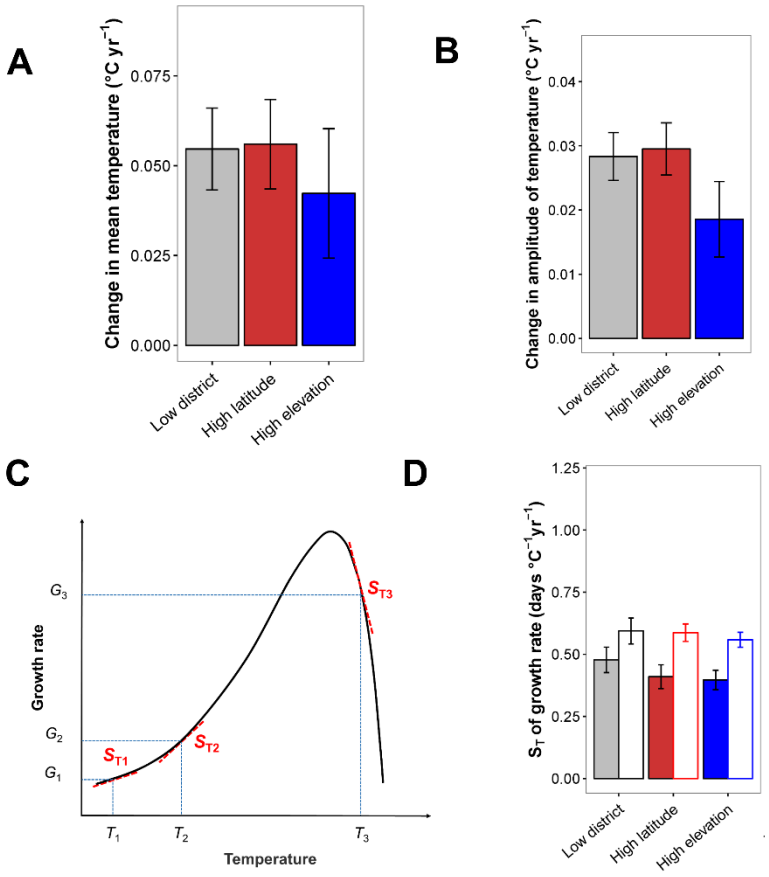

Figure 2. Temporal pattern of temperature regime and physiological response of odonates during 1980-2013 for three time windows. **A.** Change in mean temperature of the best time window. **B.** Change in amplitude of temperature of the best time window. **C.** Theoretical thermal performance curve showing the sensitivity of growth rate to temperature at three different temperatures. This plot shows that at different parts of the curve, an increase of environmental temperature (warming) could increase slightly (S_{T1}), considerably (S_{T2}) or either decrease (S_{T3}) growth rate. **D.** Change in growth response to average temperature of the best time window from 1980-1996 (filled bars) to 1997-2013 (open bars). Growth rate was estimated with thermal performance curves of nine species (Supplemental material). Error bars are 95% confidence intervals.

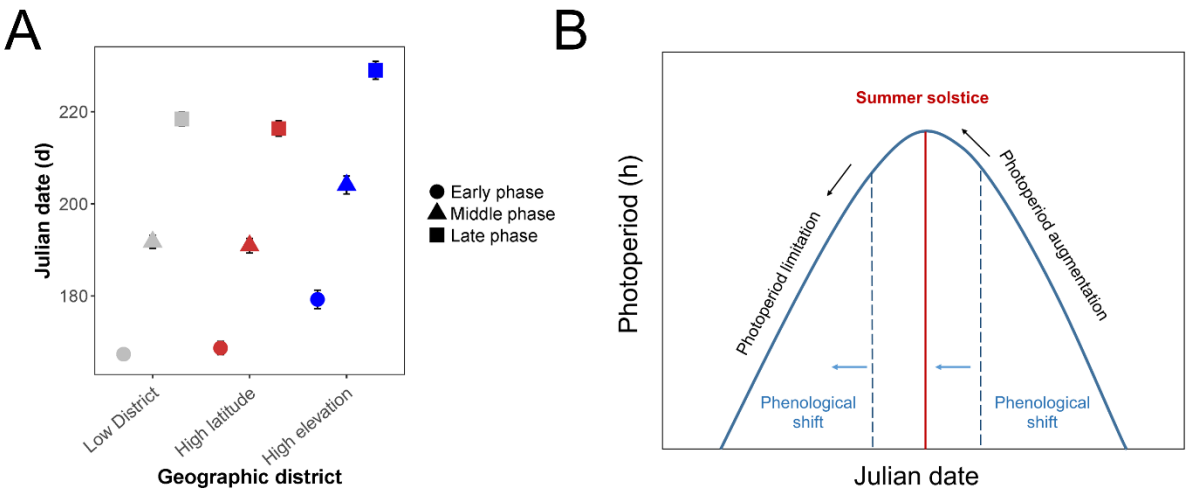

Figure 3. A. Geographic pattern of the early phenological phase of odonates. **B.** Theoretical change of photoperiod resulting from phenological shift. Whether the phenology is before or after the summer solstice (red vertical line), the photoperiod may either decrease (photoperiod limitation) or increase (photoperiod augmentation).

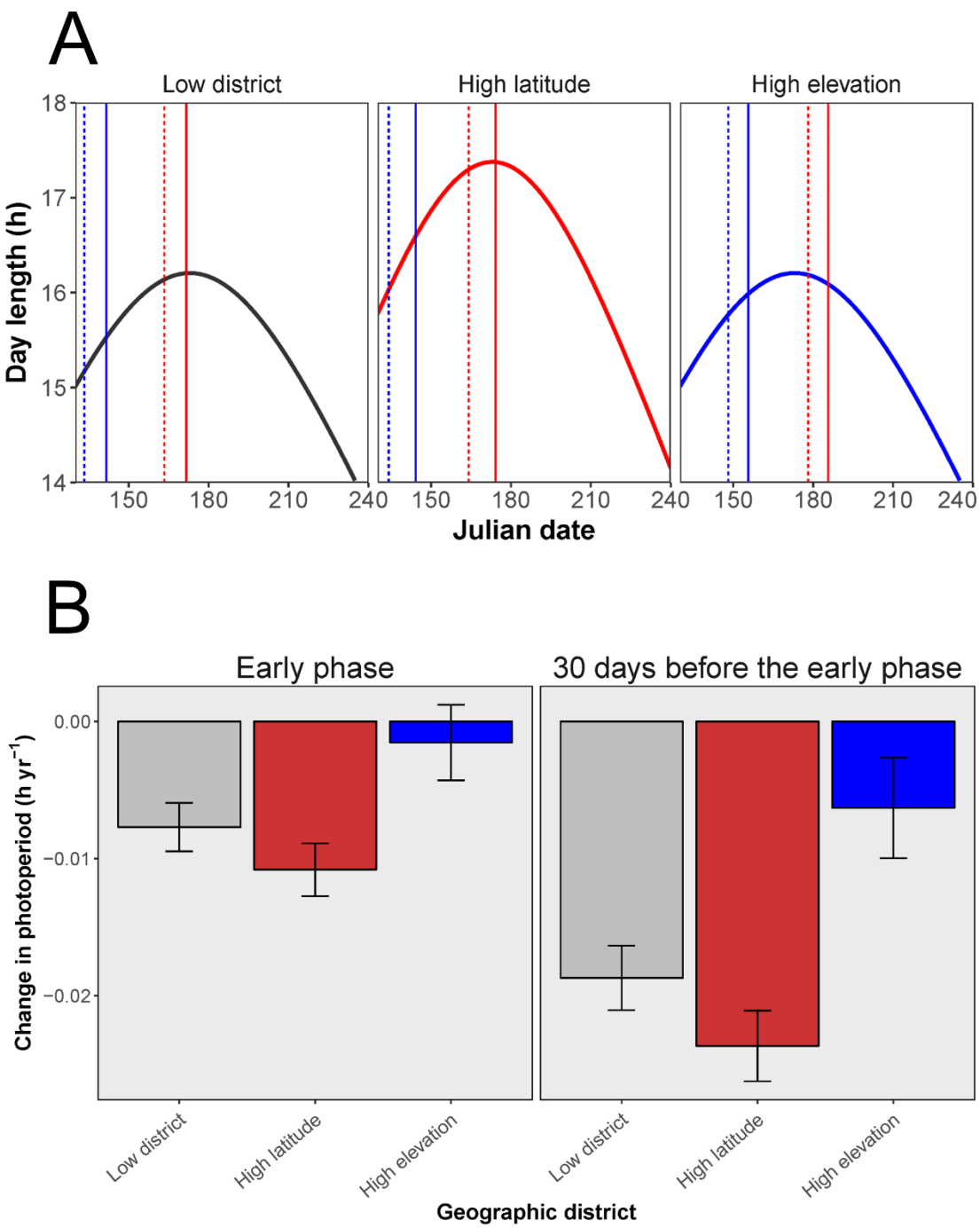

Figure 4. A. Seasonal pattern of photoperiod in the three districts with the average of the early phase of phenology during 1980-1996 (solid line with blue ribbon) and 1997-2013 (dashed line with red ribbon). **B.** Change in photoperiod during 1980-2013 in the three districts. Error bars are 95% confidence intervals.

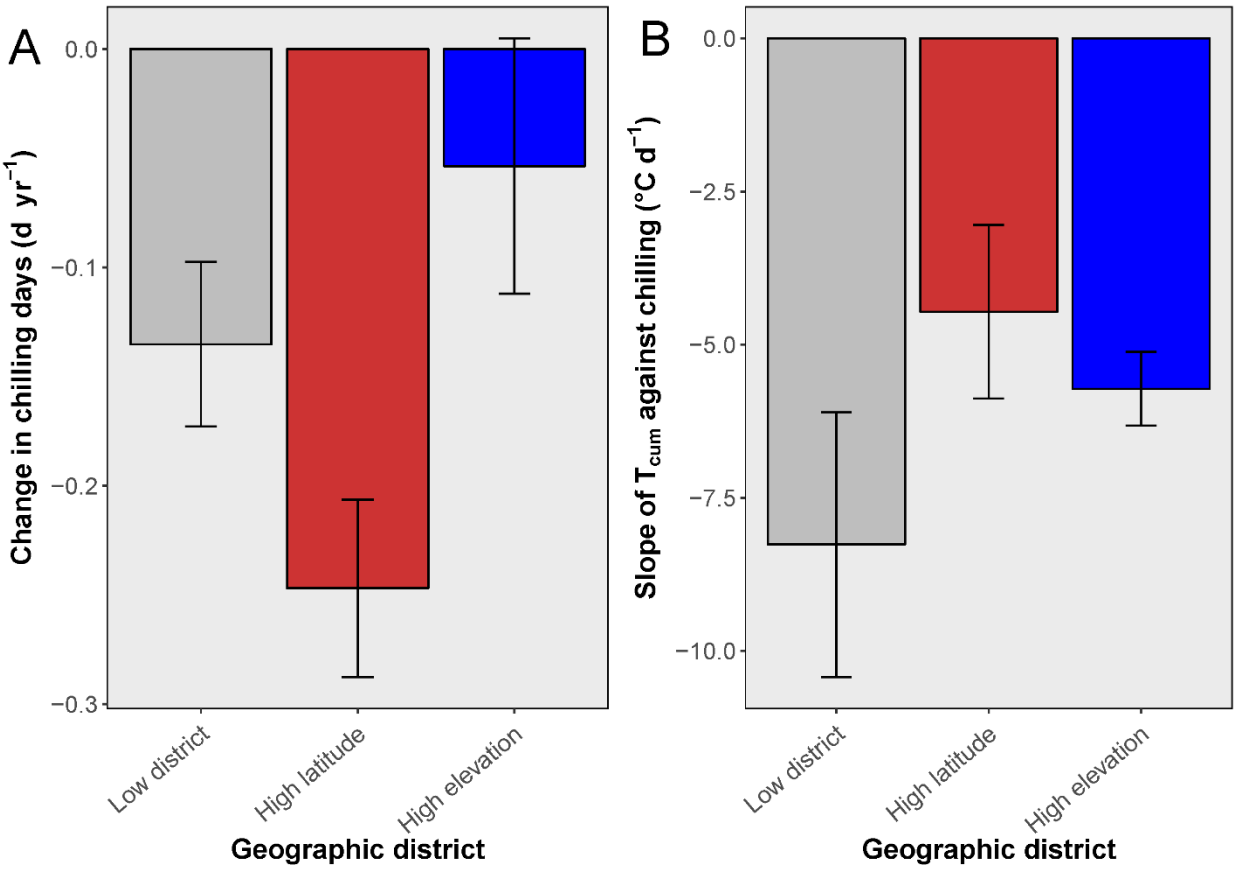

Figure 5. A. Temporal pattern of the number of chilling days during 1980-2013. **B.** Relationship of chilling requirements with the cumulative temperature needed before emergence of adults. Error bars are 95% confidence intervals.

Table 1. Summary results of the linear mixed effect model assessing the temporal pattern of S_T between 1980-1996 and 1997-2013 across latitude and elevation.

Random effects					
Groups	Name	Variance	Std.Dev.		
Species	(Intercept)	1.074	1.036		
Residual	38.800	6.229			
Fixed effects					
	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	1.834e-01	1.418e-01	5.500e+01	1.293	0.201489
z.Tmean	-2.610e-01	1.723e-02	3.856e+03	-15.152	< 2e-16
geo_High lat	-8.327e-03	2.016e-02	3.855e+03	-0.413	0.679628
geo_High Elev	2.656e-01	7.307e-02	3.855e+03	3.635	0.000282
Period[1997-2013]	-3.664e-01	1.503e-02	3.855e+03	-24.377	< 2e-16
z.Tmean: geo_High lat	-1.031e-01	2.917e-02	3.855e+03	-3.535	0.000412
z.Tmean: geo_High Elev	1.961e-01	6.232e-02	3.855e+03	3.146	0.001668
z.Tmean:Period[1997-2013]	6.840e-02	1.920e-02	3.856e+03	3.563	0.000372
geo_High lat:Period[1997-2013]	-1.567e-02	2.222e-02	3.855e+03	-0.705	0.480750
geo_High Elev:Period[1997-2013]	8.938e-02	8.087e-02	3.855e+03	1.105	0.269102
z.Tmean: geo_High lat:Period[1997-2013]	1.355e-01	3.210e-02	3.856e+03	4.221	2.49e-05
z.Tmean: geo_High Elev:Period[1997-2013]	-5.237e-02	6.744e-02	3.855e+03	-0.776	0.437508

Table 2. Summary results of the linear mixed effect model assessing the temporal pattern of photoperiod during (a) and 30 days before (b) the early phase (15th percentile) during 1980-2013.

a	**Random effects**					
	Groups	Name	Variance	Std.Dev.		
	Species	(Intercept)	0.1530	0.3911		
	Residual		0.1302	0.3608		
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	3.777e+01	1.989e+00	3.865e+03	18.989	< 2e-16
	Year	-1.053e-02	9.951e-04	3.860e+03	-10.583	< 2e-16
	geo_Low_District	-6.731e+00	2.677e+00	3.856e+03	-2.514	0.0120
	geo_High_latitude	-1.943e+01	3.453e+00	3.858e+03	-5.626	1.98e-08
	Year: geo_Low_District	2.864e-03	1.340e-03	3.856e+03	2.137	0.0326
	Year: geo_High_latitude	9.224e-03	1.728e-03	3.858e+03	5.339	9.88e-08
b	**Random effects**					
	Groups	Name	Variance	Std.Dev.		
	Species	(Intercept)	0.8994	0.9483		
	Residual		0.2383	0.4882		
	Fixed effects					
		Estimate	Std. Error	df	t value	Pr(>|t|)
	(Intercept)	6.417e+01	2.694e+00	3.873e+03	23.817	< 2e-16
	Year	-2.412e-02	1.347e-03	3.857e+03	-17.909	< 2e-16
	geo_Low_District	-1.127e+01	3.622e+00	3.855e+03	-3.111	0.00188
	geo_High_latitude	-3.660e+01	4.673e+00	3.856e+03	-7.833	6.22e-15
	Year: geo_Low_District	5.186e-03	1.813e-03	3.855e+03	2.860	0.00426
	Year: geo_High_latitude	1.801e-02	2.338e-03	3.856e+03	7.706	1.64e-14

Table 3. Summary results of the simple linear model assessing the temporal pattern of the number of chilling days during 1980-2013.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	312.86508	38.37030	8.154	4.75e-16
Year	-0.13520	0.01922	-7.036	2.34e-12
geo_High latitude	219.66016	56.45400	3.891	0.000102
geo_High elevation	-152.80805	70.88895	-2.156	0.031178
Year:geo_High latitude	-0.11176	0.02827	-3.953	7.85e-05
Year:geo_High elevation	0.08157	0.03548	2.299	0.021553

Table 4. Summary results of the linear mixed effect model assessing the relationship between post-winter cumulative temperature and the number of chilling days during 1980-2013.

Random effects					
Groups Name	Variance	Std.Dev.			
Species (Intercept)	156018	395.0			
Residual	23431	153.1			
Fixed effects					
	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	1.266e+03	5.410e+01	1.270e+02	23.40	<2e-16
Chill	-1.149e+00	4.310e-02	6.126e+05	-26.66	<2e-16
geo_High Lat	2.712e+02	8.450e+00	6.126e+05	32.09	<2e-16
geo_High Elev	1.627e+03	2.930e+01	6.126e+05	55.54	<2e-16
Chill: geo_High Lat	-2.753e+00	5.927e-02	6.126e+05	-46.45	<2e-16
Chill: geo_High Elev	-1.165e+01	1.976e-01	6.126e+05	-58.93	<2e-16

2.3. Supplementary information

The species list for phenology analysis and thermal performance curve estimation are similar to that of Chapter I (Supplementary information).

2.3.1. Latitude-for-elevation model of temperature similarity

The equivalence of temperature between latitude and elevation was calculated first by performing a simple linear regression of temperature by latitude (*Lat*) and temperature by elevation (*Elv*) in order to obtain the slope which indicates the speed at which temperature changes over the gradient, and second by dividing one slope by the other; in this case the slope of latitude by that of elevation. Due to the fact that elevation is confounded with latitude (i.e. all high elevation areas are restricted to the Alps in Switzerland), a latitudinal and elevational cut-off was carried out on the data (565 m for latitude and 52° for elevation) to divide the gradients:

$$T_1 = a_1 \times Lat + b_1 \qquad \text{knowing that } Elv < 200 \text{ m} \qquad (1)$$

$$T_2 = a_2 \times Elv + b_2 \qquad \text{knowing that } Lat < 49° \qquad (2)$$

where T_1 and T_2 is the annual mean air temperature of each location over the period 1980-2013, a_1 and a_2 are the slope (temperature lapse) and b_1 and b_2 are the intercept of the regression for latitude and elevation, respectively.

$$z = \frac{a_1}{a_2} \qquad (3)$$

where z is the temperature lapse ratio between latitude and elevation.

The results of the simple linear regression showed that a_1 = -0.446 (b_1 = 31.1, F = 153.5, DF = 49 , R^2 = 0.75) and a_2 = -0.005 (b_1 = 12.05, F = 1072, DF = 63, R^2 = 0.94). Thus, using equation (3) z = 94.3 m.$^{\circ-1}$.

Using this temperature lapse ratio, we divided the latitudinal and elevational gradients into three areas (districts) starting from the lowest geographic point of our data (46°N, 192 m): low district (46-52° N and elevation < 565.8 m), high latitude (52-58° N and elevation < 565.8 m), and high elevation (565.8-1131.6 m and latitude < 52°N) (Fig. 1A).

Table S1. Summary statistics of the fixed effects of the linear mixed effects model assessing the interaction of year and temperature on the early phase of phenology of odonates.

	Estimate	**Std. Error**	**df**	**t value**	**Pr(>|t|)**
(Intercept)	1.918e+02	3.602e+00	5.400e+01	53.259	< 2e-16
zTmean	-2.801e+00	8.560e-02	1.487e+05	-32.726	< 2e-16
zyear	-4.965e+00	4.113e-02	2.955e+05	-120.718	< 2e-16
zTmean:zyear	1.699e-01	2.905e-02	2.957e+05	5.847	5.02e-09

CHAPTER III

The use of thermal performance curves to predict development under variable lab and field conditions

Rassim Khelifa, Wolf U. Blanckenhorn, Jeannine Roy, Patrick T. Rohner, Hayat Mahdjoub

Thermal performance curves (TPC) have been estimated experimentally in multiple temperate ectotherm species using constant temperatures, but their reliability for predicting thermal responses in the wild where temperature fluctuates diurnally and seasonally remains poorly documented. Here we use distant latitudinal populations of five species of sepsid flies (Diptera: Sepsidae) from the temperate region (Europe, North Africa, North America) to compare estimates derived from constant TPCs with observed development rate under fluctuating temperatures in the laboratory and the field. TPCs changed across gradients with higher latitude or elevation flies showing faster development, an adaptive response. TPCs were then used to predict development rates observed in the laboratory and the field under fluctuating temperatures. Predictions of observed development rate were relatively accurate in the laboratory but not in the field. Interestingly, the precision of TPC-predictions depended not only on the resolution of temperature data, with diurnal and overall temperature summing apparently performing better than hourly temperature summing, but also on the frequency of temperatures falling below the estimated critical temperature minimum. Hourly temperature resolution most strongly underestimated actual development rates because flies apparently did not stop growing when temperatures fell below this threshold, thus most severely (and accurately) reflecting this error. Our results suggest that when flies do not encounter cold temperatures, TPC-predictions based on constant temperatures can accurately reflect performance under fluctuating temperatures if properly adjusted for non-linearities, but when they encounter cold temperatures this method is more error prone. Our study emphasizes the importance of the resolution of temperature data and cold temperatures in shaping thermal reaction norms, thus improving predictions of the responses of ectotherms to climate change.

Keywords: Altitude, latitude, climate change, chilling, thermal adaptation, temperature variation, Sepsidae, temperate region

3.1. Introduction

Increases in world temperature are considered a fundamental factor influencing global biodiversity by affecting species' distribution, physiology and ecology (Hughes 2000, Parmesan and Yohe 2003, Andrew et al. 2013, Nooten et al. 2014) . Ectotherms, the most diverse organisms on earth (Wilson 1992), are particularly susceptible to changes in the environmental conditions, since all of their physiological and biological functions depend on temperature (Jarošík et al. 2002, Jarośík et al. 2004, Paaijmans et al. 2013). The biological and ecological responses of organisms to increasing temperature have already been demonstrated in various organisms showing either plasticity (Charmantier et al. 2008, Seebacher et al. 2015), shifting phenology (Parmesan and Yohe 2003, Root et al. 2003, Thomas et al. 2004), or adaptation to the new environmental conditions (Hoffmann et al. 2003). Global warming is occurring differently on earth (Walther et al. 2002) and, by consequence, there is geographic variation of its impact on species. Indeed, world temperature is expected to rise by 1° to 4°C in the next 100 years (IPCC 2014). Understanding the thermal response of species to this change is therefore crucial to predict their fate in the future to inform conservation practice.

To reliably predict the response of organisms to future warming, a good understanding of their thermal adaptation and the relevant thermal parameters to which species respond is needed. Previous studies on warming effects typically considered average temperatures for spimplicity, but more recently temperature fluctuations have been shown to be important for accurately estimating the spatio-temporal thermal responses of temperate species (Bozinovic et al. 2011, Thompson et al. 2013, Vasseur et al. 2014). Although there is a great body of literature that experimentally estimated the response of species to constant temperatures (Scheiner 2002), the reliability of these estimates to predict responses to fluctuating temperatures, which clearly better reflect natural conditions at least in temperate climes, has yet to be tested.

The thermal performance curve (TPC) is an important tool to understand thermal adaptation and physiology of organisms. TPCs are usually estimated across a range of relevant constant temperatures and has a shape of a negatively skewed distribution with null performance at the critical minimum (CT_{min}) and maximum (CT_{max}) temperatures and a peak at the optimal temperature (T_{opt}), which is closer to CT_{max} (Deutsch et al. 2008). From CT_{min} to T_{opt}, the performance increase is quasi exponential. This part of the curve is very important because it includes the majority of temperatures that are encountered by most organisms in their natural

environment. Due to the nonlinearity of TPC and to the fact that temperature in the wild varies daily and seasonally, the average performance under fluctuating temperatures is not equal to the performance at the corresponding average temperature (Ruel and Ayres 1999, Vasseur et al. 2014), a mathematical property known as Jensen's Inequality. Although TPCs derived from constant temperatures have been regularly used to determine the responses of organisms to fluctuating temperatures, assuming or suggesting that the overall response is the product of the additive responses to temperatures (Liu et al. 1995), experimental studies are needed to confirm this under laboratory and especially field conditions.

TPCs vary across geographic gradients in ectotherms (Sinclair et al. 2016). Higher latitude and higher elevation sites have short warm periods during the year, shifting the TPC of species horizontally towards a lower range of temperatures (implying e.g. faster development at lower temperatures), or vertically towards higher rates implying faster development at higher latitudes and elevations at any given temperature (Yamahira et al. 2007). This suggests that a species' temperature sensitivity may evolve in response to environmental conditions. For instance, Kingsolver et al. (2007) showed that the TPC of cabbage while butterflies (*Pieris rapae*) evolved after range expansion in North America. However, evolutionary adaptation can involve various traits (Angilletta 2009), such that the absence of differences between geographically distant populations for a certain trait may hide adaptive compensatory processes in other traits (Blanckenhorn and Demont 2004). Common garden experiments are useful tools to reveal such geographic adaptations (Angilletta 2009). Here we investigate geographic patterns of TPCs across latitude and elevation in temperate dung flies.

The relevance of TPC in predicting the response of species to warming in the wild remains questionable, however. One reason is that TPCs are typically estimated under controlled laboratory conditions, which do not reflect the actual natural conditions where a large spectrum of factors may affect the involved physiological responses (Cook et al. 2012). For instance, it has been widely investigated in plants that there are clear carry-over effects of winter temperatures on spring development rate and phenology (Porter and Gawith 1999, Cook et al. 2012, Fu et al. 2015) such that spring development is usually fast after cold winters (chilling fulfillment) and relatively slow after warm winters (chilling loss). Although this topic has received so far little attention in insects, there are evidence that post-winter development is faster after longer winters (accumulating more chilling: (Bosch and Kemp 2003, Stålhandske et al. 2014)). If this pattern is general among insects,

it is reasonable to suggest that TPCs for development rate estimated under controlled laboratory conditions would not yield reliable estimates of the phenology under natural conditions featuring cold temperatures. Here we test this hypothesis with a field experiment comparing observed and TPC-predicted development time.

In this study, we estimated the TPC and response to constant and fluctuating temperature of five species of dung flies (Diptera: Sepsidae) in an experimental study, using multiple populations from distant latitudinal and elevational locations to test for potential geographic variation in thermal physiology. We here focused on development rate as a measure of performance because of its life history implications (Gillooly et al. 2002) and its relationship to fitness (Dmitriew 2011) and adult phenology (Tauber et al. 1986). Specifically, we assessed the relationship between the predicted (from constant TPC) and observed development time in the laboratory and the wild to determine the reliability of TPC in estimating the observed response to temperature.

3.2. Methods

3.2.1. Study organisms

Sepsid flies are widespread scavengers that depend on dung to breed (Fig. 1). Females lay eggs into faeces of large vertebrates, mostly cattle. Larvae are coprophagous and live within the dung, whereas adults feed on nectar of flowering plants and dung. They represent good model organisms for studies of thermal adaptations (Berger et al. 2013, Berger et al. 2014). Egg-to-adult development time varies among species from 1-6 weeks under field conditions (Pont and Meier 2002).

We performed field and laboratory experiments with five species of the genus *Sepsis*: *S. cynipsea* (Linnaeus, 1758), *S. thoracica* (Robineau-Desvoidy, 1830), *S. punctum* (Fabricius, 1794), *S. neocynipsea* (Melander & Spuler, 1917), and *S. fulgens* (Meigen, 1826). Populations used in the experiment originated from Europe, North Africa and North America (Table S1). For each species, we used two populations per species except for *S. punctum*, for which we used four. Each pair of populations was selected from latitudinal (*S. cynipsea*, *S. thoracica*, *S. punctum*), elevational (*S. punctum*, *S. neocynipsea* and *S. fulgens*) or transcontinental distant locations (*S. neocynipsea*).

3.2.2. Sample preparation

Prior to the experiments, flies were collected in the field and brought to the laboratory. Single females were isolated into plastic containers with dung provided as egg laying medium. When offspring of the isofemale-line emerged, they were kept under constant conditions (temperature 18°C, 60% humidity and 16L:8D photoperiod) in a climate chamber for multiple generations, with sugar and water *ad libitum* as food sources and fresh cattle dung for breeding.

To perform our experiments, we out-crossed 5 isofemale lines for each population and species to avoid inbreeding depression, incerase the genetic variation and reduce maternal carry-over effects by randomly mixing 40 offspring individuals (20 males and 20 females) from each isofemale-line for two generations in plasctic countainers (20×5×5 cm) at the above conditions.

3.2.2.1. Experiment I - *Estimation of thermal performance curves for development rate at constant temperatures*

Thermal performance for each population of the five species was estimated at seven constant temperatures (12°, 17°, 22°, 27°, 32°, 34° and 35°C) and constant photoperiod (16L:8D) in separate climate chambers. Temperatures were chosen using prior knowledge about *Sepsis* thermal adaptation (Blanckenhorn 1999) and the theoretical shape of the thermal performance curve. The interval between the first five temperatures (12° - 32°C) is 5 °C because these temperatures fall within the gradually increasing range of the curve, with 12°C being close to the putative lower thermal threshold; however, the thermal interval was reduced to 2° and 1°C beyond 32°C where performance was expected to decrease abruptly.

For each species and population, we supplied a replicate group of 20-30 flies in a given replicate container with food and fresh dung in a dish for oviposition. After 24 h, the dung dish (containing eggs) was removed, held in a round glass vial (10×3 cm), and checked daily for emergence of adult offspring. The position within the climate chamber of replicates, populations and species was randomized. In total, we had 9 replicates for each population, species and temperature treatment combination. Development time was estimated as the number of days from egg laying to the first emergence. We measured the effect of temperature and latitude on development rate, which was calculated as the inverse of development time.

3.2.2.2. Experiment II – *Assessment of development rate at fluctuating temperatures*

For each species and population, we exposed flies in separate climate chambers to three treatments with the same average temperature, 60% humidity and 16L:8D photoperiod. We set one treatment at constant 18°C, and two fluctuating treatments with a mean of 18°C and an amplitude of 15-21°C (± 3°C) and 11-25°C (± 7°C), respectively. Else the same methodology used in the previous experiment to raise flies was also applied here, again assessing development rate. Here we had 10 replicates for each population, species and treatment.

3.2.2.3. Experiment III – *Assessment of development rate under field conditions at the end of the season*

A field experiment assessing development rate of the same five species was conducted on the Irchel campus of the University of Zurich towards the end of the season 2016. In this experiment, all species were collected from two wild populations in Switzerland (*S. neocynipsea* from Sörenberg and all the others from Zürich). Starting in mid-August (12.08.2016) until late November, wild-caught (parental) flies were kept in 3L-containers with sugar, water and dung *ad libitum*. There were four replicate containers per species, which were placed outside in randomized order in a largely shaded location to avoid the risk of death due to overheating. Fresh cow dung was supplied (i.e. replaced) twice a week in a 100-ml cup for oviposition. When eggs were detected in the dung, the cups were isolated into a separate container at the same field site and checked daily for emergence of F1 offspring adults to estimate development time under the ever-decreasing field temperatures in autumn. We obtained corresponding hourly and daily average air temperature data of a nearby meteorological station (4 km from the field experiment) from IDAweb (https://gate.meteoswiss.ch/idaweb/) to assess the effect of fluctuating field temperatures on development rate.

3.2.3. Statistical analyses

Statistical analyses were performed with R 3.2.2 (R Development Core Team 2017). All mixed-effects models were fitted with the R package *lme4* (Bates et al. 2015). Values are mean ± SD (unless indicated).

3.2.3.1. Experiment I

To assess the effect of temperature and latitude on development rate (i.e. the inverse of development time), we used generalized mixed-effects Poisson regression with temperature and

latitude as fixed effects and species as random effect. In this analysis, temperature treatment of 34° and 35°C were excluded because no fly emerged.

To estimate the TPC at constant laboratory conditions for each population and species, temperature was used as continuous variable and all seven temperature treatments were included. Since all individuals died at 34° and 35°C, development rate was set to zero. We fitted the O'Neill function (Spain 1982). This function estimates three of the four components of the response to temperature (T_{opt}, CT_{max}, Q_{10}) (equation 1). The model assumes that growth increases with temperature with a coefficient Q_{10} prior to the optimal temperature (T_{opt}), where development is maximal (r_{max}), and decreases abruptly afterwards until the critical maximum temperature (CT_{max}) where development is no longer possible.

$$\Phi(T) = r_{max}\left(\frac{CT_{max}-T}{CT_{\max}-T_{opt}}\right)^{p}\exp\left(\frac{p(T-T_{opt})}{T_{max}-T_{opt}}\right) \quad (1)$$

Where *T* is the ambient temperature, $p = \frac{1}{400}J^2\left(1+\sqrt{1+\frac{40}{J}}\right)^2$, $J = (Q_{10}-1)\left(T-T_{opt}\right)$, and φ(T) is the respective mean development rate estimated from experimental data.

The critical minimum temperature CT_{min} was estimated as $-intercept/slope$ of the linear regression of development rate across three temperature ranges in the quasi-linear left-hand part of the TPC (Blanckenhorn 1999): 12-17°C, 12-22°C and 12-27°C. We chose different temperature ranges because the quasi-linear left-hand part of the TPC is actually exponential and thus the slope changes somewhat with an increase in temperature. By pooling data from different species and populations, we tested for relationships between the various CT_{min} estimated for 12-17°C, 12-22°C and 12-27°C with Spearman correlation tests.

3.2.3.2. Experiment II

Linear mixed-effects Poisson regressions were again performed for each species to assess the effect of temperature fluctuation treatment (18°, 18±3° and 18±7°C) and geographic location (low and high latitude or elevation) on development rate. To assess the usefulness of the TPC estimated with constant temperatures (as in experiment I) in predicting the actually observed performance under fluctuating temperature, we derived estimates based on hourly temperature readings (in the climate chambers) to predict development rate obtained from our laboratory experiment. We estimated the hourly development rate by dividing the TPC-estimated development rate by 24 h

(since the unit of TPC is day). Then we summed all hourly development rates to obtain the predicted daily development rate. Because the same frequency distribution of temperatures is used every day in the laboratory experiment, the mean development rate for the entire development time is theoretically identical to that for any given day.

To directly compare the observed and TPC-predicted development rate, reduced major axis regressions (observed development rate being the response) assessing deviations from unity were conducted for each species using the lmodel2 R package (Legendre 2014).

3.2.3.3. Experiment III

For each species, the relationship between TPC-predicted and field-observed development rate was assessed at three different resolutions of temperature measurements with reduced major axis regression (observed development rate being the response), as above testing for potential deviations from unity. The three temperature scales were hourly average, daily average and overall average temperature (average of daily temperatures across the entire development).

To test for the potential relationship between the precision of the estimated development time and frequency of cold conditions (i.e. temperatures below estimated CT_{min}), we fitted a linear mixed-effects model with the ratio of observed-by-predicted development time as the response variable, the number of days below CT_{min} as a fixed effect, and species as a random effect. The slope of the relationship between field-observed and TPC-estimated development time was further estimated separately for flies that did not encounter cold conditions (no temperatures below CT_{min}) and those that experienced cold conditions (number of days below CT_{min}).

3.3. Results

3.3.1. Effects of constant laboratory temperatures on development and TPC

Development time differed among species (Fig. 2a). From fastest to slowest, respective mean ± SD development time across all viable temperatures (from 12° to 32°C) and populations were: *S. cynipsea* (19.57±12.00 d, n=206), *S. thoracica* (20.81±11.98 d, n=274), *S. punctum* (23.59±12.29 d, n=569), *S. neocynipsea* (25.61±14.54 d, n=198), and *S. fulgens* (23.83±13.09 d, n=311) (Table S2). Development time was significantly negatively related to temperature (Fig. 2a; Table 1). The fastest change in development time was recorded between 12° and 17°C with an average decline (across species) of 0.60 [95%CI: 0.57-0.62] $d°C^{-1}$. In all species, flies did not reach the adult life

stage at 34° and 35°C, thus assuming that the former is the critical maximum temperature. Interestingly, development time was affected by elevation similarly at all temperatures (shorter when higher; Fig. 2b; Table 1). In contrast, development time decreased with latitude only for the three highest temperature treatments 22°, 27° and 32°C.

Development rate was estimated as the inverse of development time, and the thermal performance curve was then fitted by the O'Neil function using seven temperatures: 12, 17, 22, 27, 32, 34 and 35°C (Fig. 3). Our model showed that T_{opt}, r_{max} and Q_{10} varied across species (Table S3). T_{opt} varied between 29.9°C in *S. punctum* and 31.5°C in *S. thoracica*; r_{max} (maximal development rate) ranged between 0.10 d^{-1} in *S. fulgens* and 0.17 d^{-1} in *S. thoracica*; and Q10 varied between 1.7 in *S. punctum* and 1.82 in *S. cynipsea*. There was a significant positive relationship between r_{max} and T_{opt} (Fig. 4; linear model: r = 0.03, SE= 0.007, P = 0.002), showing that species that have larger optimal temperature tend to have faster development rate.

CT_{min} consistently increased with increasing temperature range, yielding an average across species of 6.49°C [95%CI: 6.32-6.67] for 12-17°C, 7.64°C [95%CI: 7.36-7.91] for 12-22°C, and 7.85°C [95%CI: 7.50-8.19] for 12-27°C. CT_{min} estimated with 12-22°C was positively correlated with that estimated with 12-27°C (Spearman's rank correlation: r = 0.86, P = 0.0005), but the other pairwise correlations were not significant (range: r =0.30-0.55; P > 0.05).

3.3.2. Relationship between observed and predicted development rate in the laboratory

All species responded similarly to temperature fluctuations: the greater the fluctuation, the faster the development rate (Fig. 5, Table 2). On average, low (18±3°C) and high fluctuating (18±7°C) temperature increased development rate by 4.3% and 12.9%, respectively. However, neither latitude nor elevation affected the development rate (Table 2).

When pooling all species, populations and treatments, the slope of the relationship between experimentally observed and TPC-based predicted development rate was slightly but not significantly different from unity (slope = 0.97 [0.92 - 1.03], R^2 = 0.97, N = 36), revealing that estimates derived from constant TPCs are close to those observed even when temperature fluctuates (Fig. 6). A separate analysis for each species also showed no significant deviations from unity in four species: *S. cynipsea* (slope = 0.86 [95%CI: 0.59–1.24], R^2 = 0.93, N = 6), *S. punctum* (slope = 0.98 [95%CI: 0.77–1.22], R^2 = 0.90, N = 12), *S. fulgens* (slope = 0.77 [95%CI: 0.57–1.03], R^2 = 0.96, N = 6), *S. thoracica* (slope = 0.86 [95%CI: 0.44–1.53], R^2 = 0.85, N = 6); only in

S. neocynipsea a significant deviation was observed (slope = 0.69 [95%CI: 0.50–0.94], $R^2 = 0.95$, N = 6). The same analysis for each temperature fluctuation treatment equally showed no deviations from unity: 18°C (slope = 1.01 [95%CI: 0.95–1.06], $R^2 = 0.99$, N = 12), 18±3°C (slope = 1.04 [95%CI: 0.96–1.13], $R^2 = 0.98$, N = 12) and 18±7°C (slope = 0.99 [95%CI: 0.87–1.12], $R^2 = 0.97$, N = 12). Note that the power to detect deviation was low in these latter, separate analyses.

3.3.3. Relationship between observed and predicted development rates in the field

Mean ± SD development times observed in the field experiment towards the end of the season varied within and across species: 26.86±12.06 d (n=84) for *S. cynipsea*, 32.66±17.15 d (n=84) for *S. thoracica*, 34.68±13.27 d (n=84) for *S. punctum*, 35.38±17.44 d (n=84) for *S. fulgens*, and 36.35±17.70 d (n=63) for *S. neocynipsea*. We estimated the development rate from the (constant) TPC and field temperatures at three different resolutions: overall average development rate, daily average, and hourly average. Although the relationship between the field-observed and TPC-based predicted development rate differed significantly from unity at all scales (Fig. 7), diurnal and overall average temperature showed slopes closer to 1:1 (0.95 [95%CI: 0.93 - 0.97]) than hourly temperature (1.20 [1.16 - 1.25]). This suggests that under field conditions, laboratory-estimated TPCs tend to underestimate development rate when using hourly temperatures, whereas estimation became more accurate at lower resolutions.

While the performances of daily and overall average temperature in predicting the observed development rate were similar, they showed some differences for two species (Table S4). Daily average temperature yielded better prediction for *S. cynipsea* with a slope of 0.98 [95%CI: 0.93-1.04] compared to 0.94 [0.9-0.99], whereas overall average temperature gave better prediction for *S. neocynipsea* with a slope of 0.98 [0.95-1.01] compared to 0.92 [0.88-0.96].

We calculated the prediction precision as the ratio of observed over predicted development rate (ratio = 1 means perfect prediction) for the hourly, daily average and overall temperature to find that, across species, the ratio was 1.09 [95%CI: 1.08 – 1.10] for the hourly average temperature, 1.12 [95%CI: 1.10 – 1.14] for the daily average temperature, and 1.02 [1.01 – 1.03] for the overall average temperature (during development time), revealing that the latter resolution provided better predictions. Nevertheless, prediction precision was negatively related to the number of chilling days (the number of days below CT_{min}) at daily and overall average temperature resolutions for all three CT_{min} estimations (Fig. 8), unsurprisingly showing highest prediction

reliability when the number of chilling days was zero (Table 3: intercept ≈ 1); as chilling days became more frequent towards the end of the season, the predicted development rate became lower than observed (observed/predicted ratio > 1). When dividing individuals into two categories – those that encountered temperatures that fell below CT_{min} (CT_{min1}; chilled individuals) and those that did not (non-chilled individuals) – we were able to reveal that for non-chilled individuals, daily average temperature performed better than the hourly and overall average temperature at predicting the observed development rate, showing a slope of 0.98 [0.97 – 0.99] compared to 1.10 [1.08-1.12] and 0.93 [0.92-0.94], respectively. The inverse was true for chilled individuals, for which the overall average temperature performed better (1.04 [1.02-1.05]) than the hourly (1.09 [1.07-1.11]) and daily average temperature (1.15 [1.12-1.18]).

3.4. Discussion

Whether thermal performance curves (TPC) of ectotherms estimated with constant temperatures can be used to predict performance at naturally fluctuating temperatures is fundamental and timely (Angilletta 2009, Sinclair et al. 2016). In this study, we combined laboratory and field experiments on five species of *Sepsis* flies to address three main questions focusing on development time (or rate) as a performance measure because of its important phenological and fitness implications (Gillooly et al. 2002) (Dmitriew 2011): (1) Do fluctuating temperatures affect the estimate of development rate? (2) Do TPCs estimated with constant temperatures correctly predict the effects of temperature fluctuations under laboratory conditions? (3) Do (constant) TPCs predict the effects of temperature fluctuations under natural field conditions? We found that fluctuating temperatures below the optimal temperature positively affect development rate, and also demonstrated that TPCs derived from constant temperatures yield reliable estimates of development time and rate under laboratory conditions when corrected, but this technique has limitations for predicting development under natural conditions when winter approaches.

TPCs for development rate of five common sepsid flies were typical for ectotherms. We observed significant differences among species, with *S. cynipsea* and *S. thoracica*, the most common species in north-central and southern Europe (respectively), showing fastest development at warm temperatures (22-32°C), and *S. fulgens* showing the slowest development. These interspecific differences at least partly relate to these flies' distribution range, with the faster developing species being warm-adapted (and common in southern temperate region) and slower

developing species being cold-adapted or widespread (*S. punctum, S. fulgens*, *S. neocynipsea*) (Pont and Meier 2002). In all five species, we documented an optimal temperature (T_{opt}) for development rate around 29-32°C that was positively correlated to their maximum development rate, suggesting that fast developing species are more warm-adapted, although we could not detect any significant interspecific differences in their critical maxima. However, because of the typically steep decline of the TPC towards the critical maximum beyond T_{opt} (Fig. 3), further detailed studies scrutinizing potential interspecific differences at temperatures between 32° and 34°C are needed to reveal possible adaptions of the upper thermal extreme (CT_{max}) at the species and population levels.

In addition to these interspecific differences, populations of most species varied geographically in their responses to constant laboratory temperatures along elevation and latitude. Extending Berger et al.'s (2014) study of *S. punctum,* flies consistently developed faster at higher elevations across the entire range of temperatures (12-32°C), whereas they developed faster at higher latitudes only at the warmer temperatures used here (22-32°C), revealing adaptation to cold climes across both gradients. Faster development in cooler regions with short seasons is adaptive because flies can complete their life cycle faster at any given (cool) temperature, and thus likely explains the observed systematic changes of thermal reaction norms at higher latitudes and elevations found here (Homeny and Juliano 2007, Yamahira et al. 2007, Kipyatkov and Lopatina 2010, Mikolajewski et al. 2015). The differences in the geographic response pattern between latitude and elevation may be explained by the steeper temperature decline along elevation and the concomitant stronger selection gradient (Pickett 1989). Thus, higher elevation populations (maximum here = 1350 m) are likely exposed more to colder temperatures than high latitudinal populations (maximum = 49.5°).

Theoretically, temperature fluctuations below the optimal temperature (T_{opt}), where the TPC is convex and increases exponentially, should speed up development relative to the corresponding constant temperature, and this effect should become stronger as fluctuations increase, as found here (see 18±7°C vs. 18±3°C in Fig. 5; (Ruel and Ayres 1999). Similar results were e.g. observed in the mosquito *Anopheles stephensi* (Paaijmans et al. 2013). According to Jensen's inequality (see Introduction), opposite effects result when the fluctuations extend to the concave section of the TPC to the right of T_{opt}, as observed e.g. in the cold-adapted yellow dung fly (Kjaersgaard et al. 2013). These results suggest that the phenology of adults may shift more

strongly than expected based on mean temperatures in regions where warming increases thermal extremes.

Our main objective was to verify the predictive power of TPCs estimated from constant temperatures to data generated at fluctuating temperatures. To confirm this hypothesis, we here used an approach that compares development rates predicted from constant TPC with the actually observed development rates generated under (1) controlled laboratory conditions with specific diurnally fluctuating temperatures, and (2) erratically fluctuating field conditions towards the end of the season. We found that TPC predicts the observed development rate relatively well when the above-mentioned non-linear effect (Jensen's inequality) is properly adjusted for by rate summation (Liu et al. 1995). Else, when assuming constant as opposed to fluctuating temperatures, development times and rates can be considerably under- or overestimated (Fig. 5).

When applied to field conditions, this rate-summing method may be used to project the effect of climate warming in the future (Vasseur et al. 2014). However, our field experiments showed that the accuracy of TPC-based predictions of development rate was limited and quite complex. As the accuracy of rate summing must depend on the resolution of the temperature data, we applied three temperature resolutions including hourly, daily and overall average temperatures to test which gives most reliable estimates of pre-winter development. Interestingly and unexpectedly, and in contrast to our laboratory experiments, daily and gross average temperatures yielded more accurate predictions, whereas hourly resolution generally most severely underestimated actual development rates (Fig. 7; Fig. 8). Not surprisingly perhaps, the predictability of field development rates towards the end of the season here depended on the frequency of cold temperatures below the estimated critical minimum temperature CT_{min}. The latter here hovered around 6 – 7°C for all species (Table S3), and more cold days (or hours) encountered in the field led to greater underestimation (i.e. individuals emerged earlier than expected; Fig. 8). When excluding individuals that encountered temperatures below their critical minimum, predictions were more reliable, showing that not accounting for cold conditions (chilling) underestimates (here) pre-winter development, potentially leading to erroneous predictions and misleading conclusions. Similar findings in other insects also indicate that the number of chilling days positively affects (i.e. advances) the day of spring emergence (Bosch and Kemp 2003, Stålhandske et al. 2014). Cold temperatures seem to advance development by increasing the thermal sensitivity of flies, ultimately leading to earlier emergence before winter

frost might kill the juveniles (adaptive explanation). Alternatively, the standard method for estimating the lower temperature threshold (e.g. Blanckenhorn 1999; see Methods) may generally overestimate CT_{min}, meaning that flies do *not* stop growing after all when temperatures fall below this threshold, thus generally leading to underestimated growth rates (non-adaptive explanation). Hourly temperature resolution most strongly underestimates actual development rates because often temperatures fall under the putative lower threshold only for some hours during any given day, thus most severely (i.e. accurately) reflecting this error. Since we think that this pattern of underestimated pre-winter (and perhaps also post-winter) growth at cold temperatures might be general among ectotherms, it is essential that future attempts to predict climate change effects on phenology must consider the potential effect of cold temperatures (ranging below critical minimum) on the thermal responses of species, before or after entering diapause (Stålhandske et al. 2014, 2017).

In recent years, increasing attention has been paid to the importance of temperature fluctuations for predicting the thermal and phenological responses of temperate ectotherms (Vasseur et al. 2014). It is by now clear that organisms are well adapted to fluctuating temperatures, and that by using constant temperatures in laboratory experiments we may actually miss something, in the extreme reaching wrong conclusions. Our study adds a new layer of information regarding the usefulness and limitation of TPCs for predicting organisms' responses to climate change by combining theoretical, laboratory and field data. We confirmed that fluctuating temperatures alter development rate also in sepsid flies, mandating that future studies of thermal responses of life history traits should consider naturally variable temperatures to obtain more accurate estimates better reflecting those observed in the wild. We also confirmed that TPCs derived from constant temperatures can reasonably be used to predict thermal responses to fluctuating temperatures under controlled laboratory conditions if properly adjusted. However, when applying the same approach to field data, predictions derived from constant TPCs can be less reliable, particularly when temperatures below the critical lower temperature threshold are involved. We showed that predictions and estimations further depend on the resolution of temperature data (hourly, daily or gross average), and that considering different thermal scales relevant for the study organisms is essential. As seasonal and diurnal temperature fluctuations in the temperate region often span the left-hand region of TPC, crossing the critical minimum temperature, climate change is likely to alter the thermal sensitivity of species, which makes long-

term projections of the future phenology of ectotherms using TPCs quite difficult. The next challenge to urgently tackle would be the understanding of the relationship between cold conditions and thermal sensitivity of development (Stålhandske et al. 2017).

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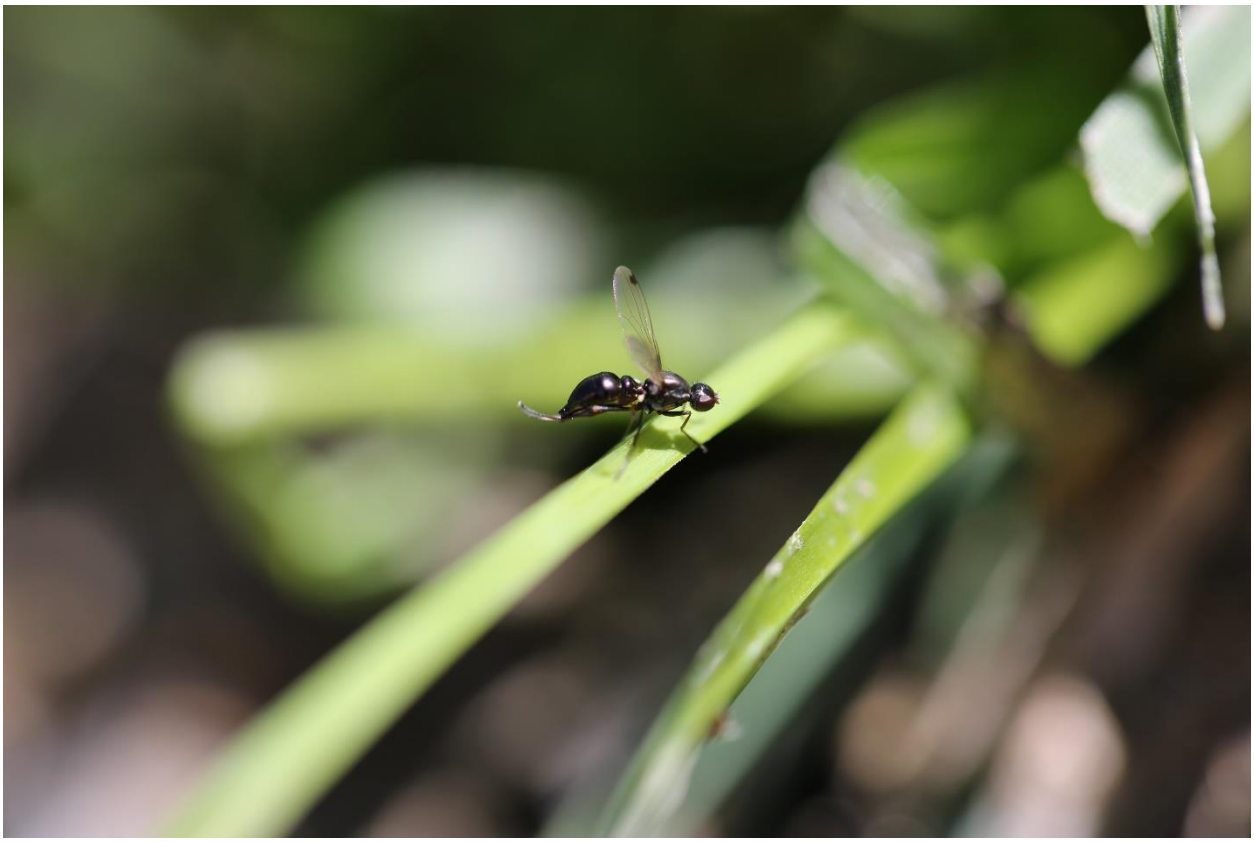

Figure 1. Adult female *Sepsis* sp. in the wild (Flumserberg 2016, Rassim Khelifa).

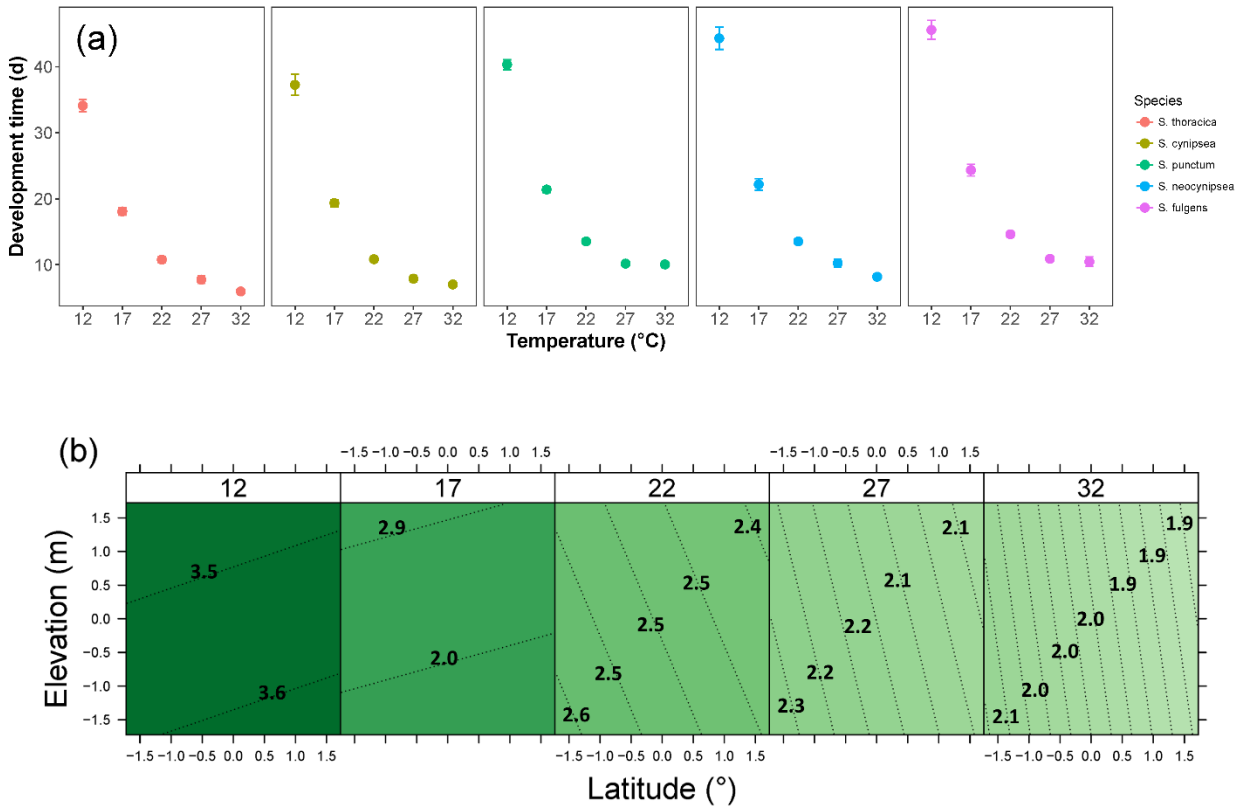

Figure 2. (a) Development time of five *Sepsis* species at five viable temperature treatments (12°, 17°, 22°, 27°, 32°C). Error bars are 95% confidence intervals. (b) The effect of latitude (standardized), elevation (standardized) and temperature on development rate. Values on contours are predictions of standardized development time from generalized mixed-effects Poisson models. The smaller the value (the lighter the color), the earlier the adult emergence.

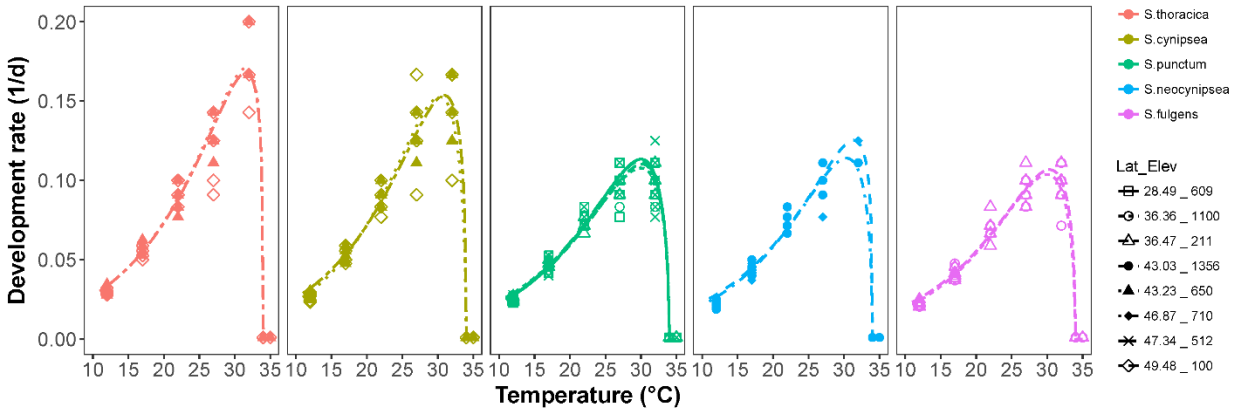

Figure 3. Thermal performance curves (TPC) for development rate of five species of *Sepsis* at seven temperatures (12°, 17°, 22°, 27°, 32°, 34°, 35°C) as fitted by the O'Neil function. Parameter estimates for each species are shown in Table S3.

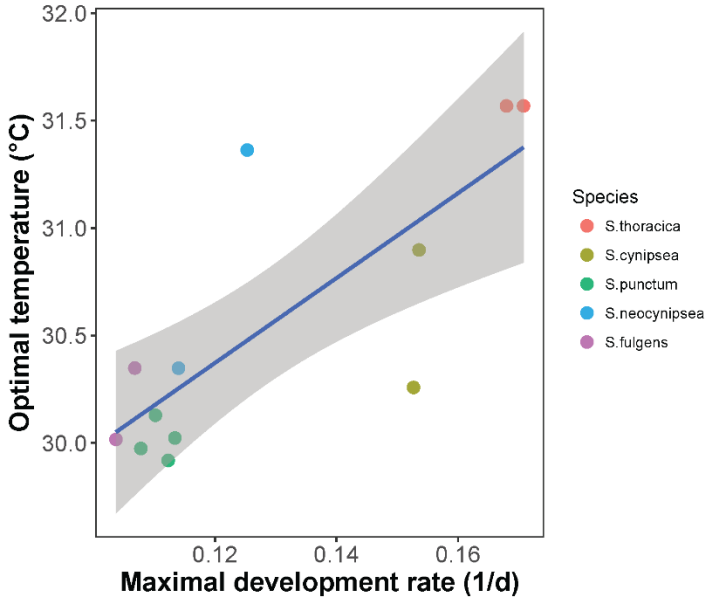

Figure 4. Relationship between optimal temperature and maximal development rate of five *Sepsis* species (2 – 4 populations per species). The fitted line is a linear regression with 95% confidence intervals and significantly positive (r = 0.031, SE= 0.007, t = 4.027, P = 0.002), showing that faster developing species have warmer optimal temperature.

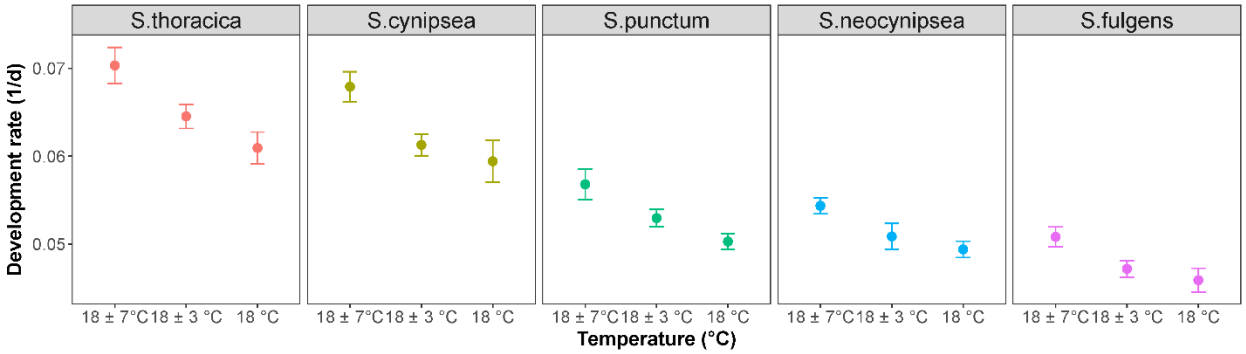

Figure 5. Mean ± SD effect of diurnal temperature fluctuation on the development rate of five *Sepsis* species. The three temperature correspond to a constant (18°C), a relatively variable (15-21°C) and a highly variable temperature (11-25°C) treatment with an average of 18°C.

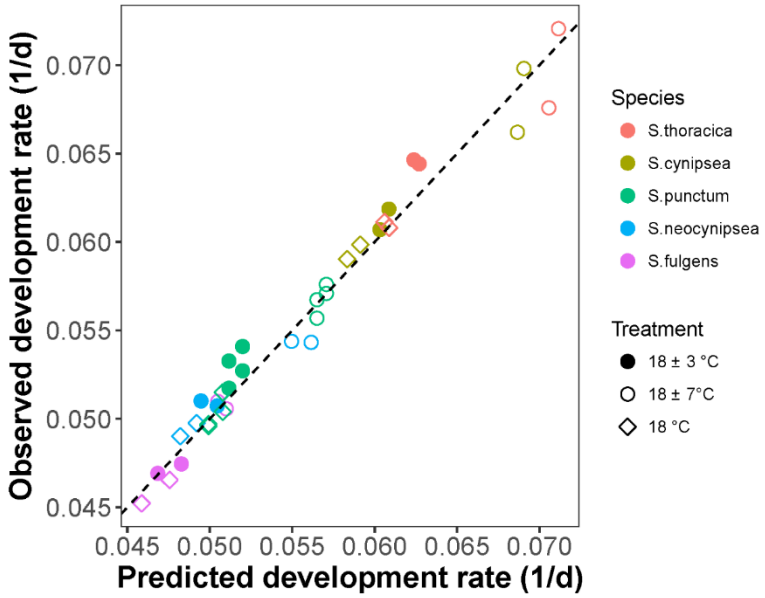

Figure 6. Relationship between predicted and observed development rate estimated in the laboratory. Predicted development rates were estimated from the thermal performance curve using hourly temperature measurements. The diagonal line has a slope of 1.

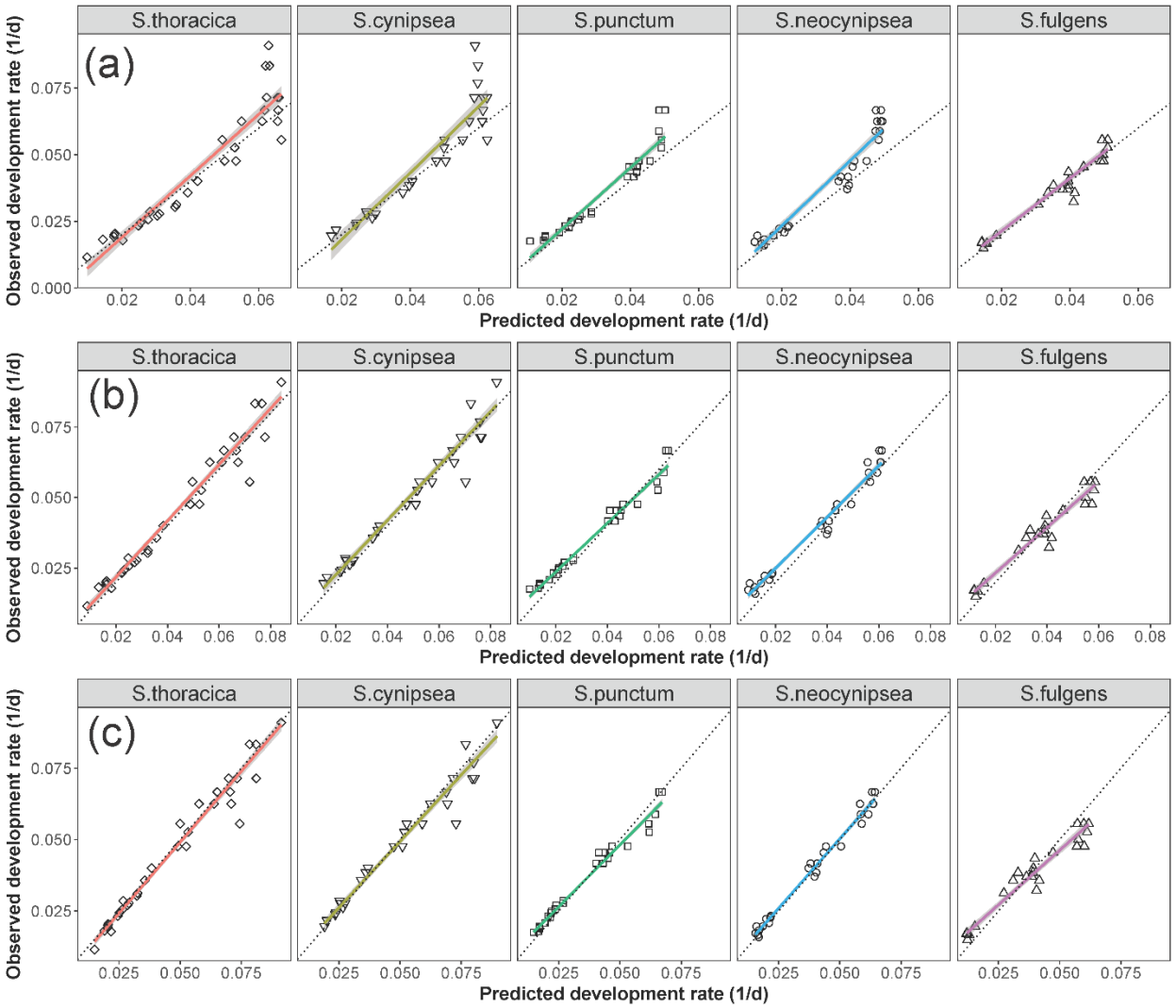

Figure 7. Relationship between predicted and observed development rate estimated in the field. Predicted development rate was estimated using hourly average temperature (a), daily average temperature (b), or overall average temperature (c). The diagonal line has a slope of 1, and the fitted line is a simple linear regression.

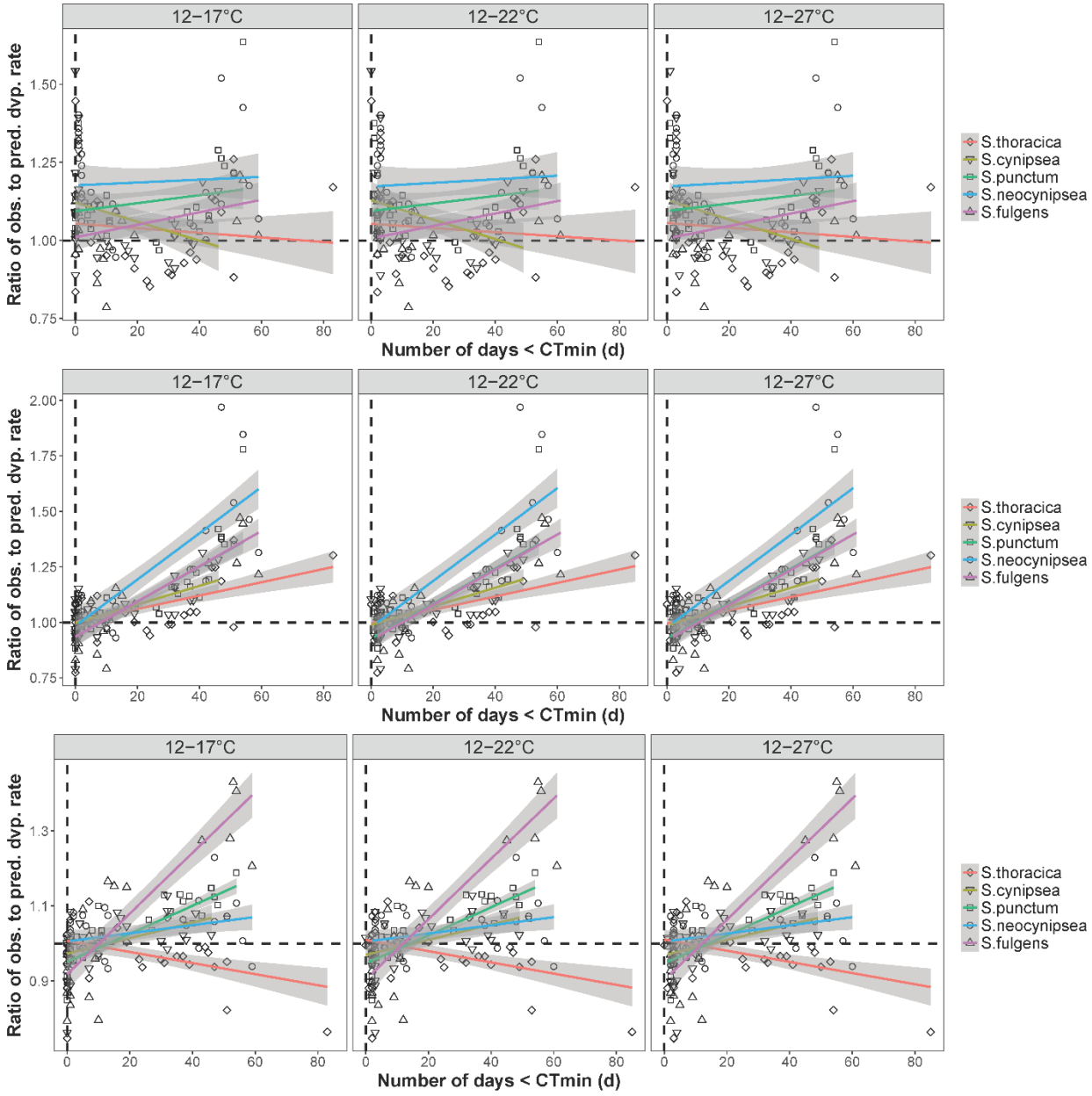

Figure 8. Relationship between the ratio of observed-to-predicted development rate and the number of days below the estimated critical minimum temperature CT_{min} (see Table S3) for hourly average temperature (a), daily temperature (b), and for the overall average temperature (c). The three panels represent three estimates of CT_{min} (see Methods). When the ratio > 1, the observed values are underestimated by the (constant) thermal performance curve, whereas when the ratio < 1, the observed values are overestimated. The fitted lines are simple linear regressions.

Table 1. Summary statistics of the generalized mixed-effects Poisson regression testing for the effect of temperature, elevation and latitude on development time of five *Sepsis* species (N = 1486). The intercept is temperature treatment 12°C.

Fixed effects		**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
	(Intercept)	3.740	0.052	71.380	< 2e-16
	Temp[17]	-0.600	0.013	-44.940	< 2e-16
	Temp[22]	-1.045	0.016	-66.890	< 2e-16
	Temp[27]	-1.382	0.022	-63.950	< 2e-16
	Temp[32]	-1.555	0.031	-49.440	< 2e-16
	z.Latitude	0.004	0.010	0.450	0.653
	z.Elevation	-0.014	0.007	-2.130	0.033
	Temp[17]:z.Latitude	-0.001	0.013	-0.060	0.949
	Temp[22]:z.Latitude	-0.036	0.016	-2.280	0.022
	Temp[27]:z.Latitude	-0.058	0.022	-2.610	0.009
	Temp[32]:z.Latitude	-0.096	0.036	-2.660	0.008

Table 2. Summary statistics of the linear mixed-effects regression testing for the effects of temperature fluctuation and geographic location on development rate of five *Sepsis* species (N = 313).

Fixed effects		**Estimate**	**Std. Error**	**df**	**t value**	**Pr(>|t|)**
	Intercept[18°C]	0.053	0.003	4.030	15.691	< 2e-16
	Temperature[18 ± 3°C]	0.002	0.000	304.60	4.965	< 2e-16
	Temperature[18 ± 7°C]	0.007	0.000	304.60	14.26	< 2e-16
	z.Latitude	0.000	0.000	306.50	0.623	0.534
	z.Elevation	0.000	0.000	305.70	1.281	0.201

Table 3. Summary results of the linear mixed-effects model testing for the effect of cold conditions on the ratio of observed-to-predicted development rate of five *Sepsis* species using hourly average temperature (a) daily average temperature (b) or overall average temperature (c) and three CT_{min} estimates (N= 267). CT_{min1}, CT_{min2} and CT_{min3} were calculated using linear regression across the following temperature ranges: 12-17°C, 12-22°C and 12-27°C, respectively (see Methods).

CT_{min}	Resolution		Estimate	Std. Error	df	t value	Pr(>|t|)
CT_{min1}	**(a) Hourly**	(Intercept)	0.9303	0.0241	4.8600	38.6790	<0.0001
		No.days < CTmin	-0.0001	0.0003	262.0000	-0.2720	0.7860
	(b) Daily	(Intercept)	1.0210	0.0179	4.9600	57.1700	<0.0001
		No.days < CTmin	-0.0048	0.0003	262.1000	-19.260	< 2e-16
	(c) Overall	(Intercept)	1.0280	0.0167	5.4300	61.4570	<0.0001
		No.days < CTmin	-0.0019	0.0003	262.6000	-7.0610	<0.0001
CT_{min2}	**(a) Hourly**	(Intercept)	0.9307	0.0242	4.9800	38.4640	< 2e-16
		No.days < CTmin	-0.0001	0.0003	261.8000	-0.3150	0.7530
	(b) Daily	(Intercept)	1.0270	0.0188	4.9700	54.5900	< 2e-16
		No.days < CTmin	-0.0048	0.0003	261.8000	-19.260	< 2e-16
	(c) Overall	(Intercept)	1.0294	0.0168	5.6500	61.1510	< 2e-16
		No.days < CTmin	-0.0019	0.0003	262.2200	-6.9190	< 2e-16
CT_{min3}	**(a) Hourly**	(Intercept)	0.9305	0.0242	5.0000	38.3790	< 2e-16
		No.days < CTmin	-0.0001	0.0003	261.8000	-0.2750	0.7840
	(b) Daily	(Intercept)	1.0280	0.0193	4.9500	53.280	< 2e-16
		No.days < CTmin	-0.0048	0.0003	261.8000	-19.160	< 2e-16
	(c) Overall	(Intercept)	1.0300	0.0171	5.6400	60.2260	< 2e-16
		No.days < CTmin	-0.0019	0.0003	262.2000	-6.8650	< 2e-16

3.6. Supplementary information

Table S1. Geographic coordinates and elevation of study populations of the five study species.

Species	Country	Site	Geographic location (gradient)	Latitude (°)	Longitude (°)	Elevation (m)
S. thoracica	Germany	Ludwigshafen	High latitude	49.48	8.42	100
S. thoracica	Italy	Petroia	Low latitude	43.23	12.56	650
S. punctum	Switzerland	Zurich	High latitude	47.34	8.54	512
S. punctum	Spain	La Laguna (Tenerife)	Low latitude	28.49	-16.31	609
S. cynipsea	Germany	Ludwigshafen	High latitude	49.48	8.42	100
S. cynipsea	Italy	Petroia	Low latitude	43.23	12.56	650
S. punctum	Algeria	Maouna	High elevation	36.36	7.41	1100
S. punctum	Algeria	Old Bridge	Low elevation	36.47	7.37	211
S. fulgens	Algeria	Maouna	High elevation	36.36	7.41	1100
S. fulgens	Algeria	Old Bridge	Low elevation	36.47	7.37	211
S. neocynipsea	Switzerland	Sörenberg	High latitude	46.87	8.27	710
S. neocynipsea	USA	Fort Hall (ID)	Low latitude	43.03	-112.44	1356

Table S2. Development time averaged across all temperature treatments.

Species	Population	Mean	n	sd	LCI	UCI
S. cynipsea	Ludwigshafen	21.45192	104	12.59132	19.03199	23.87185
S. cynipsea	Petroia	17.40000	90	10.94809	15.13814	19.66186
S. fulgens	Maouna	24.16779	149	13.71742	21.96523	26.37034
S. fulgens	Old Bridge	23.48667	150	12.48179	21.48920	25.48413
S. neocynipsea	Fort Hall	24.19540	87	13.88980	21.27674	27.11407
S. neocynipsea	Sorenberg	26.84848	99	15.05254	23.88338	29.81359
S. punctum	La laguna	24.71739	138	13.22461	22.51095	26.92383
S. punctum	Maouna	21.99174	121	11.90203	19.87105	24.11242
S. punctum	Old Bridge	24.07097	155	12.19970	22.15039	25.99154
S. punctum	Zurich	23.32824	131	11.70236	21.32430	25.33219
S. thoracica	Ludwigshafen	21.60000	155	12.06132	19.70121	23.49879
S. thoracica	Padula	19.66355	107	11.81456	17.42497	21.90214

Table S3. Parameter estimates for the thermal performance curves of five species of *Sepsis*. r_{max} (maximum development rate), T_{opt} (optimal temperature), CT_{max} (maximum critical temperature) and Q_{10} were estimated with the O'Neil function, whereas CT_{min} was estimated with a linear model (see Methods).

Species	Geographic location	r_{max}	T_{opt}	CT_{max}	Q_{10}	CT_{min}
S. fulgens	High elevation	0.10	30.02	34.00	1.75	6.23
	Low elevation	0.11	30.35	34.00	1.73	6.36
S. cynipsea	High latitude	0.15	30.26	34.00	1.82	6.45
	Low latitude	0.15	30.90	34.00	1.76	6.73
S. thoracica	High latitude	0.17	31.57	34.00	1.74	6.41
	Low latitude	0.17	31.57	34.00	1.75	6.35
S. neocynipsea	High latitude	0.11	30.35	34.00	1.74	7.21
	Low latitude	0.13	31.36	34.00	1.71	6.74
S. punctum	High latitude	0.11	29.92	34.00	1.73	6.01
	Low latitude	0.11	30.02	34.00	1.74	6.71
	High elevation	0.11	29.97	34.00	1.70	6.37
	Low elevation	0.11	30.13	34.00	1.72	6.38

Table S4. Slope estimation of the relationship between field-observed and TPC-based predicted development rate using reduced major axis regression. Bold slopes differ significantly from 1.

Scale of temperature	Species	Slope	95%CI Slope
Hourly average	*S. cynipsea*	1.31	1.18-1.45
	S. fulgens	1.22	1.12-1.34
	S. punctum	1.19	1.11-1.27
	S. thoracica	1.20	1.11-1.29
	S. neocynipsea	1.25	1.17-1.35
Diurnal average	*S. cynipsea*	0.98	0.93-1.04
	S. fulgens	0.90	0.84-0.96
	S. punctum	0.87	0.84-0.91
	S. thoracica	1.01	0.97-1.06
	S. neocynipsea	0.92	0.88-0.96
Average across development time	*S. cynipsea*	0.94	0.9-0.99
	S. fulgens	0.90	0.83-0.96
	S. punctum	0.87	0.84-0.9
	S. thoracica	1.01	0.97-1.05
	S. neocynipsea	0.98	0.95-1.01

CHAPTER IV

Faking death to avoid male coercion: extreme sexual conflict resolution in a dragonfly

Rassim Khelifa

Sexual conflict is common among insects and arises from differences in the evolutionary interests of males and females. Males tend to mate with different females, whereas females do not similarly benefit from different males. In some dragonflies, males try to coerce unguarded females during oviposition and this reduces female survival. Here I document a new case of sexual death feigning as a way to avoid male coercion in a common dragonfly, the Moorland Hawker (*Aeshna juncea*). This is the fifth case of sexual death feigning for the animal kingdom. The implication of the mating system in the evolution of the behavior is discussed.

Keywords **:** Behavior, sexual selection, odonates, insect, Swiss Alps

Note :

The paper was largely covered by the media. It was reported in 39 news outlets such as *National Geographic*, *New Scientists*, *New York Post*, *Yahoo*, *Live Science*, *Science Recorder* and *Le Monde*. I had radio interviews with *BBC*, *CBC Toronto* and *CBC British Columbia* about this paper. Podcasts in *Player FM* and *Young Turk* also devoted time to talk about this new case of sexual death feigning.

Khelifa 2017. *Ecology* **98** : 1724-1726

I spent the summers of 2014 and 2015 in the Swiss Alps, collecting the eggs of odonates (dragonflies and damselflies) for laboratory experiments on larval responses to temperature. This involved many hours spent waiting beside ponds to capture females as they came to the water to lay eggs. On 5 July 2015, while I was waiting at a pond near Arosa, at about 2000 m elevation, I witnessed a dragonfly dive to the ground while being pursued by another dragonfly. I grabbed my camera and started filming (Video S1). As I approached the two insects, I realized that they were *Aeshna juncea* (sedge darner or moorland hawker), that the individual that crashed was a female, and that she was lying motionless and upside down on the ground. Upside down is an atypical posture for a dragonfly. The male hovered above the female for a couple seconds and then left. I expected that the female could be unconscious or even dead after her crash landing, but she surprised me by flying away quickly as I approached. The question arose: did she just trick that male? Did she fake death to avoid male harassment? If so, this would be the first record of *sexual death feigning* in odonates and probably the fifth in the animal kingdom after a nuptial gift-giving spider (Bilde et al. 2006, Hansen et al. 2008), two species of robber fly (Dennis and Lavigne 1976), and a European mantis (Lawrence 1992). Kaiser (1985) described motionless hiding by females in another dragonfly (*A. cyanea*; blue hawker), but did not indicate that they were faking death. I also wondered how common this behavior might be. The observation near Arosa prompted me to remember several other instances during the previous season in which I had seen dragonflies diving into the ground or vegetation. *Aeshna juncea* is common at each of the ponds where those observations occurred; could sexual death feigning occur regularly in this species?

To answer these questions, I studied the reproductive behavior of *A. juncea* for 72 hours in July and August 2015 at two sites (the Arosa pond, 46.80°N, 9.67°E and another pond near Lenzerheide, 46.73°N, 9.55°E) from 10:00 to 16:00. In both ponds, the bank vegetation, where most egg laying takes place, was relatively dense with most patches not exceeding 60 cm height. As for many other dragonflies, the female reproductive episode of the common hawker may be divided into four important sequential events: female arrival at reproductive site, copulation, oviposition, and departure from reproductive site. To summarize, mature males remained close to the water waiting for females. When the female came to the pond, the male intercepted her in the air and both formed the copulatory wheel. Copulation took place near the pond, often perched on a plant support (Fig. 1). After copulation, the male detached himself from the female and flew away. The female laid eggs (oviposition) solitarily without male protection (Fig. 1), unlike many

other dragonflies (Corbet 1999). Female became vulnerable to male coercion at that time because conspecific males were constantly patrolling each corner of the pond looking for a mate. To overcome this pressure, females showed both preventive and protective behavioral strategies to avoid coercion during oviposition and departure from reproductive sites.

To reveal potential preventive behavioral strategies of females, I estimated the vegetation density (percentage of 1 m² water area covered by vegetation) around oviposition sites of 56 and 46 ovipositing females during five days in Arosa and Lenzerheide, respectively (Appendix S1). I hypothesized that females choose sheltered areas of the pond to reduce its visibility to coercive males. On average, females laid eggs in sites with high vegetation density of 70.9% in Arosa [n = 56] and 69.2% in Lenzerheide [n = 46]. I conducted an experiment in which I reduced male density by ≥ 50 % in the two sites during one day (Appendix S1), which reduced of the average density of oviposition sites in vegetation (Appendix S1: Fig. S1a) and decreased the number of male coercion events (Appendix S1: Fig. S1b). These results suggest that males shape habitat selection of females, and thus the occupancy of densely vegetated parts of the pond during oviposition could be a behavior to reduce male coercion.

To assess the protective behavioral strategies of females during coercion, I assessed female behavior during departure from the reproductive site, which is probably the phase where the female is most vulnerable to coercion. Figure 2 illustrates behavioral responses of females to male coercion after oviposition and Table 1 presents the respective statistics for both sites. When the female tried to leave the oviposition site, males always chased her on the air. Females (n = 35) usually crashed on the ground (88.6%, n = 31), and rarely kept flying (11.4%, n = 4) (chi-square test, $P < 0.0001$) (Table 1). Females who did not crash to the ground or vegetation were all intercepted by a male. Females crashed (n = 31) landed more often within vegetation such as bushes and dense grasses (71%, n = 22), than on open areas on the ground (29%, n = 9) (chi-square test, $P = 0.02$). Following the crash, death feigning was observed in 27 out of 31 cases (87%). Of the 27 motionless females, 21 (77.7 %) were successful in deceiving the coercive male. The high frequency of sexual death feigning in both sites suggests that this behavior is common for the species. In addition, that males could not detect the motionless females highlights the importance of movement for males to detect females, which is the case for many odonates (Bick and Bick 1961, Ubukata 1984).

To test whether females are sensitive to touching during death feigning, I performed an experiment in early August 2015 at Arosa pond in which I attempted to catch by hand females performing death feigning after male harassment. Usually, it is impossible to catch an active dragonfly by hand because they rapidly escape. Of 31 catching attempts, 27 females successfully escaped (87%). Therefore, when females display death feigning they are perfectly conscious and readily avoid disturbance and probably predators.

So how did sexual death feigning evolve? On one hand, this behavior could have resulted from exaptation. Since death feigning already exists in the behavioral repertoire of dragonflies (Corbet 1999), females of the common hawker expanded the use of this antipredatory function to avoid male coercion. On the other hand, the origin of this exaptation is probably sexual conflict where each sex adopts reproductive strategies that best serve its own survival and reproductive success (Parker et al. 1979). The mating system of the common hawker is predisposed to sexual conflict because the vulnerability of solitary oviposition, the fitness costs of male harassment (Rice 1996, Crudgington and Siva-Jothy 2000), and the highly male-biased sex ratio in oviposition sites (Wildermuth 1993) put the females under extreme reproductive conditions that require effective behavioral responses to overcome survival and reproductive costs. Thus, females that perform death feigning probably undergo less coercion, survive longer and produce more offspring, hypotheses that warrant testing.

Sexual death feigning is one of the rarest behaviors in nature, and due to its scarcity, it has received little attention in behavioral ecology. Currently, it is restricted only to arthropods. It would be interesting to know whether this scarcity is true or just an artefact related to the lack of behavioral investigations or difficulty indetecting this behavior. Further studies should investigate how widespread sexual death feigning is among arthropods and whether it occurs in other phyla. Moreover, it is time to develop an informative classification for death feigning behavior that takes into account both behavioral and physiological information because death feigning could be strictly behavioral in which the animal is conscious and sensitive to touching and handling (common hawker-like), but could also include physiological changes where the animal is 'unconscious', physiologically shut down and unresponsive to physical contact (opossum-like). Finally, the new case of sexual death feigning reported here demonstrates discoveries even in common species and well-studied areas of the world remain. Although the diversity of sizes,

shapes, structures and colorations of organisms on earth is astonishingly high, the diversity of behaviors might be surprisingly comparable.

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Figure 1. Moorland hawker (*Aeshna juncea*) (Flumserberg 2016, Rassim Khelifa). Reproductive pair (left); Oviposition of a single female (right).

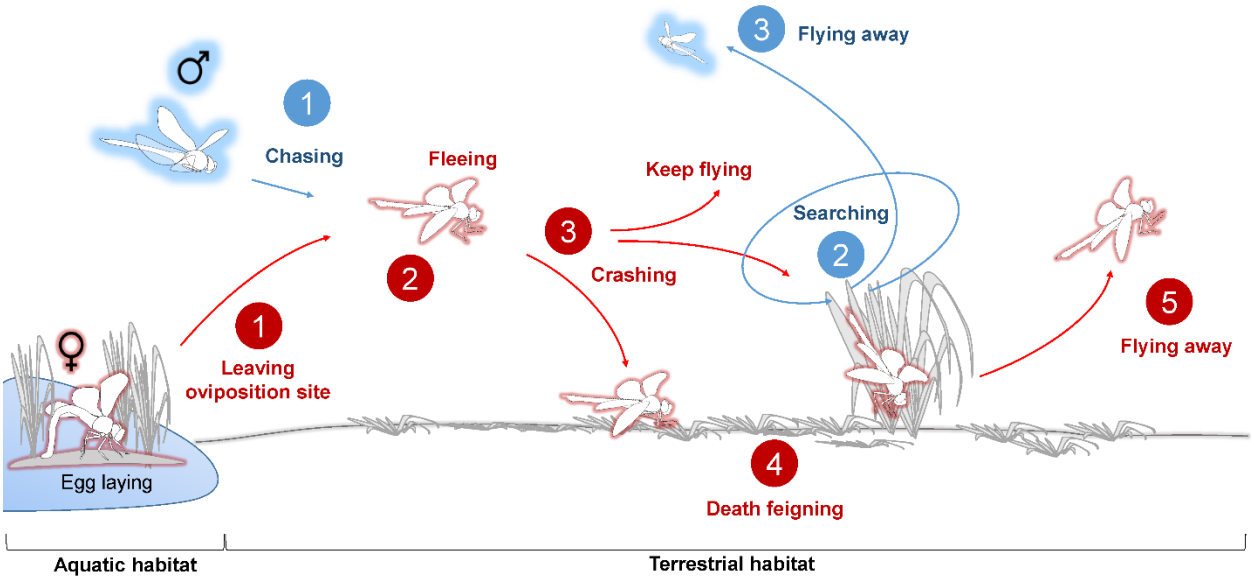

Figure 2. Death feigning of female (in red) against male (in blue) coercion of the moorland hawker (*Aeshna juncea*). Numbers refer to the succession of the events. When the female leaves oviposition site (red 1), a male usually chases her (blue 1) which induces the female to flee (red 2). The female either keep flying or crash on the ground or vegetation (red 3). She performs death feigning right after crashing (red 4) while the male is searching for her (blue 2). The male does not detect the female and leaves the crashing area (blue 3). The female, knowing that she is no longer coerced, flies away (red 5).

Table 1. Percentage occurrence of behavioral responses of females of *Aeshna juncea* to male harassment at Arosa and Lenzerheid pond sites. Values between brackets are proportions (number of observations of a behavioral response with respect to the total number of observations). Chasing response happens when the female is pursued by a male in flight. Post-crashing response happens when the female is on the ground after intentionally crashing to escape the male.

	Female behavior	**Arosa (%)**	**Lenzerheid (%)**	**Total (%)**
Chasing response	Crashing on the ground	33.3 (6/18)	17.6 (3/17)	25.7 (9/35)
	Crashing in the vegetation	44.4 (8/18)	82.3 (14/17)	62.8 (22/35)
	Flying	22.2 (4/18)	0 (0/17)	11.4 (4/35)
Post-crashing response	Death feigning	78.5 (11/14)	94.1 (16/17)	87.1 (27/31)
	Successful male deception (Male departure)	72.7 (8/11)	81.2 (13/16)	77.7 (21/27)

4.2. Video S1

Please find the link that leads you to the video:

http://onlinelibrary.wiley.com/store/10.1002/ecy.1781/asset/supinfo/ecy1781-sup-0002-VideoS1.wmv?v=1&s=50a76ac5b87b2bee45393be80a867d836a9e70f4

4.3. Appendix S1

4.2.1. Supplementary Methods

To determine site selection of females during oviposition, transects along the bank vegetation of the two sites where females lay eggs were conducted from 12:00 to 14:00 for six different occasions (days) in July and early August 2015. In these transects, vegetation density (VD) within 1 m² of the ovipositing female was estimated in percentage (the percentage of 1 m² of water covered by vegetation) with 0% corresponding to the absence of vegetation and 100% corresponding to full coverage.

To test whether females change their habitat preferences as a result of male harassment, VD was estimated after conducting a male removal experiment on the sixth occasion (day 6) in early August 2015 in both sites. The male removal experiment consisted of capturing males with a hand net during the morning (starting from around 09:00; before the appearance of females), holding them in cages for the entire day, then releasing them in late afternoon (after 16:00). The number of males was reduced by 9 and 11 individuals, which corresponded to an estimated decline of 64.3% and 55% of male abundance in Arosa and Lenzerheide, respectively. The latter percentages (P) were calculated by the following equation:

$$P = \left(\frac{N_c}{N_c + N_o}\right) \times 100$$

where N_c is number of captured males, N_o is the number of observed males recorded during the peak activity (estimated along a transect at 13:00).

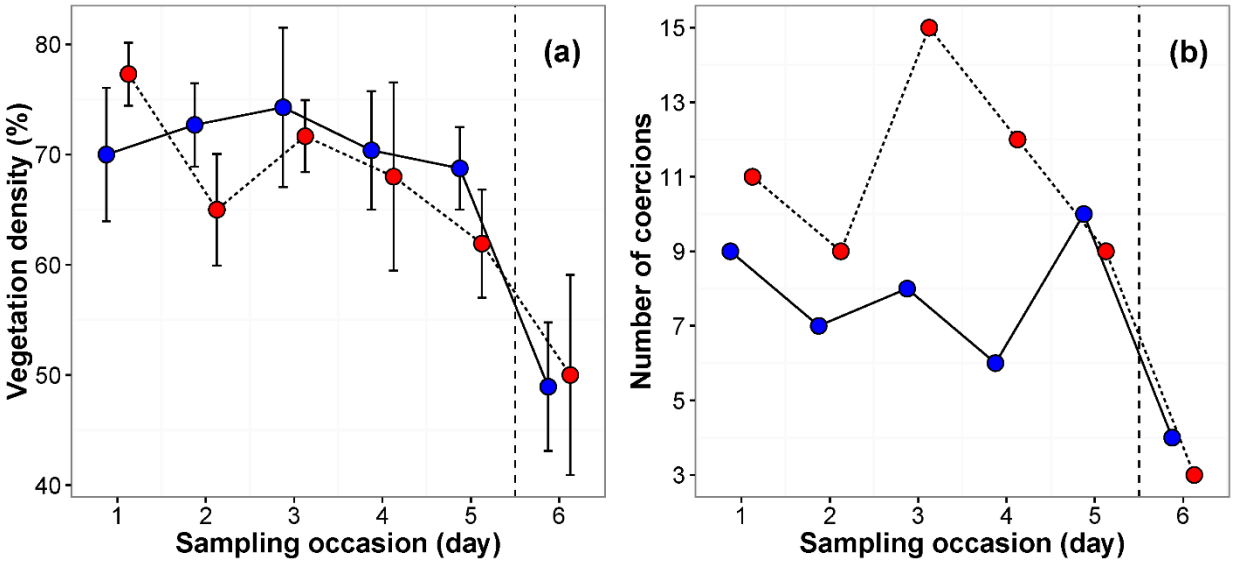

Figure S1. (a) Density of vegetation at sites used for oviposition by females and **(b)** number of cases of coercion recorded in the common hawker (*Aeshna juncea*) in Arosa (blue) and Lenzerheide (red) during six occasions. The vertical dashed line indicates the start of male removal experiment. Error bars are 95% confidence intervals. Note the decline of vegetation density of sites used for oviposition and number of coercion cases after male removal.

CHAPTER V

A century on from 'The Biology of Dragonflies' by Tillyard 1917: What have we learned since then?

Rassim Khelifa, Gunther Theischinger, Ian Endersby

The field of odonatology has developed considerably during the past century. Three figures E. Selys-Longchamps, R.J. Tillyard and P.S. Corbet have undisputedly founded our current knowledge of odonatology and contributed massively to the understanding of systematics, biology, ecology and behavior of odonates. The year of 2017 will mark the 100th anniversary of 'The Biology of Dragonflies' published by Tillyard in 1917. We review the book and the author's life and contributions to Australian odonatology. We present an updated history of odonatology and highlight prominent advances in the field. The influence of the book on non-scientists is described. Future research in odonatology on aspects that have not been studied and others that need further investigations are discussed.

Keywords: Odonata, history, systematics, biology, conservation, Australian odonatology

Khelifa et al. 2017 *Austral Entomology* **56**: 138-147

5.1. Introduction

The history of odonatology, entomologists and especially odonatologists may celebrate the 100th anniversary of one of the pioneer books in odonatology, "*The biology of dragonflies*" (Tillyard (1917a). This book is the first to compile detailed information on the morphology, anatomy, taxonomy and natural history of odonates, and thus presents the foundation of the science of odonatology. It has deeply influenced the following generations of people and scientists in their perception of these insects and increased the desire among amateurs and researchers to explore their diversity, distribution, biology, behaviour and ecology (Corbet 1962, 1999, Córdoba-Aguilar 2008). Nowadays, odonates are widely recognized as excellent model organisms with which one can answer various questions related to demography (Cordero-Rivera and Stoks 2008), evolutionary biology (Johansson and Mikolajewski 2008, Stoks et al. 2008), community ecology (McPeek 2008), and conservation biology (Samways 2008).

Here, we present (1) a short biography of Tillyard, (2) an overview of the book, (3) contribution of Tillyard to Australian odonatofauna, (4) a brief history of odonatology, (5) prominent advances in odonatology, (6) The influence of the book on non-scientists, and (7) future directions.

5.2. Short biography

Born on 31 January 1881 at Norwich, Norfolk, England, Robin John Tillyard (1881-1937) was a prominent naturalist; an entomologist with a deep interest in paleontology. He was educated at Dover College, and took up a scholarship to Queens' College Cambridge (B.A., 1903; M.A., 1907). Due to health problems he migrated to Australia and taught mathematics and science at Sydney Grammar School in 1904-13. He started publishing on odonates in 1905. His passion for studying insects and especially dragonflies led him to resign his position and study zoology at the University of Sydney. Successful in his studies, he earned a B.Sc. in 1914 and a Doctorate in Science in 1917; the year during which he published *The Biology of Dragonflies* (Tillyard 1917a) a gold mine of information for subsequent odonatologists. He was Macleay Fellow in Zoology at the Linnean Society of New South Wales in 1915-1920, and Chief of the Biological Department of the Cawthron Institute in Nelson, New Zealand in 1921-1928 where he wrote *The Insects of Australia and New Zealand* (Tillyard 1926), considered a seminal book in the history of Australian entomology. In 1928, he returned to Australia to set up the Division of Economic Entomology in

the Council for Scientific Research. From 1924 to 1937, he published several papers in paleoentomology of Australia and the Elmo, Kansas Lower Permian fossil beds (Beckemeyer 2000). He died in 1937 at the age of 56 in a car accident in Australia. More information about the life of Robin John Tillyard can be found in Imms (1938), Evans (1963), Crosby (1977), Baker (2010) and Endersby & Fliedner (2015).

5.3. Overview of the book

With 396 pages and 19 chapters (Table 1), Tillyard (1917a) established the fundamental biological knowledge of odonates based on his own research, with a particular emphasis on morphology, anatomy, taxonomy, embryology, ontogeny and phylogeny. A century later, no comparable endeavor on the biology of odonates has been published, as subsequent odonatologists did not thoroughly explore the biological aspect of odonates but used the book as basis to explore other aspects such as behaviour and ecology. Corbet (1999) stated 'The most comprehensive treatment of external and internal anatomy remains that of Tillyard (1917)'. This places the book as the definitive work on the biology of the insect group. The book contains 187 figures and four plates, two of which are coloured (e.g. Fig. 1a, b). The quality and precision of the sketches are astonishing, readily comparable with sketches made today in books and field guides using substantially advanced technology.

The first chapter is an introduction which takes the reader through the history of phylogeny of odonates, generalities on the habits and morphology of adults and larvae, and the position of the order in the Insecta. He started by acknowledging the work of marked odonatologists who contributed in the description of odonates in the 19th century, especially Baron Edmond de Selys-Longchamps and his disciples. The second chapter is devoted to the external features and exoskeleton of adults and gives detailed descriptions of the segmentation and appendages of the head, the thorax, and the abdomen. Although the chapter places emphasis on the external anatomy, it also includes an internal view of the thorax and part of the abdomen. The third chapter focuses entirely on wings, probably because they formed the basis for Odonata classification. Here, Tillyard standardizes the terminology for wing venation and presents seven figures and a plate (Fig. 1b) to illustrate the types of venation and shape diversity of different wing regions. He also writes about the wing development and phylogenetics. Chapter four is the second longest chapter and it describes the development and the external anatomy of the larval stage. He starts by giving a description of egg hatching if odonates and describes larval growth by highlighting the

morphological changes that take place during development. As in the discussion of adults, he gives detailed terminology of the various parts and segments of the larval body (head, thorax and abdomen). Tillyard made the distinction between two types of labial masks in odonates namely the *flat* and the *concave* (*spoon-shaped*) mask, and two types of dorsal appendages (*appendix dorsalis*), that is, the anal pyramid in anisopterans and caudal gills in zygopterans. The chapter ends with a description of the metamorphosis supplemented by an illustration of the different steps of the process in *Petalura gigantea*. This figure contains a controversy as it presents a hanging back emergence style (Fig. 2a) which does not correspond to the upright emergence style which seems to be the norm for the species (Fig. 2b; Baird, 2013). So, either Tillyard used the species only for illustrative purpose to describe the metamorphosis in dragonflies or he observed an unusual behaviour for the species. The seven following chapters (Chapter 5-11), which represent one third of the book, are dedicated to the physiology of the group where he describes the alimentary and excretory systems, the nervous system, the sense organs, the circulatory system, the respiratory system, the body-wall and muscles, and the reproductive system. The sketches that are presented in these chapters are accurate and reliable even today. Chapter 12 is about embryology and it presents the structure of the egg, the embryonic development, and the origin of the parts of the embryo. Chapter 13 is devoted to the coloration of odonates outlining the nature of colours, body, wing, larval coloration, and colour dimorphism. Chapter 14 presents the classification of the order with two suborders, five families 18 subfamilies and 26 tribes. In chapter 15 (zoogeographical distribution), the odonatofauna of the six main zoogeographical regions is divided into palaeogenic, entogenic and ectogenic groups. Chapter 16 is entitled geological records and presents fossil records in the palaeozoic, mesozoic, and tertiary. The author attempted to reconstruct the phylogeny of the order from the Carboniferous. Chapter 17 is on bionomics which is the only part of the book that treats natural history with some information on ecology and behaviour of odonates. Chapter 18 presents the British odonates with 42 species that Tillyard classified into 20 genera, 10 tribes, 9 subfamilies and 4 families. Chapter 19 is the last part of the book in which the author gives recommendations on how to collect and rear odonates and presents biological methods to manipulate and dissect specimens.

5.4. Contribution of Tillyard to Australian odonatology

"*The Biology of Dragonflies*" benefited greatly from Tillyard's tremendous first-hand knowledge of the Australian fauna with species, genera, even families so different from other faunas much

better known at his time. Whereas it is well-known that Tillyard introduced into science approximately as many Australian species as were previously known, considerably less attention is usually given to his taxonomic larval work that was crucial for coordinating the wealth of biological and ecological traits and making them useful for systematics and phylogeny. Tillyard was the first to discover, breed out and describe the larvae of nearly 50 species of Australian dragonflies (Table 2). His descriptions were of a standard that makes them still very useful today. He used details of more than 30 of them in '*The Biology of Dragonflies*'.

Tillyard described 87 species and 21 genera from Australia. He also named Odonata from New Zealand, Fiji, and Papua New Guinea and, if ranks lower than species are included, 25 genera and 129 specific, subspecific or infrasubspecific taxa can be attributed to him. All but two of his genera (*Anacordulia*, *Notoneura*) are still recognised, as are 52 of his species (40%). Thirty-seven (29%) of his species have been moved to another genus while 16 (12%) have fallen into junior synonymy. Twelve (9%) of his subspecies have been raised to full species status and two species have been relegated to subspecific status. Of the eleven subspecies, or varieties or races as Tillyard sometimes called them, not accounted for above, five are still recognised, albeit four in different genera, two are no longer considered as distinct subspecies, and four have disappeared from the modern literature.

Tillyard's taxonomic publishing career spanned the years 1906 to 1926 and is contained in twenty-nine articles. Twenty-three of these were published in the *Proceedings of the Linnean Society of New South Wales*. The description of the New Zealand genus *Uropetala* was, understandably, published in the *Transactions of the Royal Society of New Zealand* and his work on Fiji specimens in *Transactions of the Royal Entomological Society London*. Tillyard rarely published jointly; two exceptions are with Herbert Campion in the *Journal of the Linnean Society (Zoology)* (Tillyard 1916a) and in Aeschnines, in 'Collections Zoologiques du Baron Edm. de Selys-Longchamps' Vol XIX which was edited by its prime author René Martin (Martin, 1909).

It was very fortunate and in a way also a credit to Tillyard that some of his work on Australian dragonflies could be continued by the very prominent Australian J.A.L. Watson. Tillyard's descriptive efforts on the Australian dragonflies inspired and facilitated not only numerous detailed studies but also several comprehensive works on the fauna of this continent (e.g. Fraser 1960; Watson 1962; Watson 1991; Watson *et al.* 1991; Theischinger & Hawking 2005; Peters & Theischinger 2007; Theischinger & Endersby 2009) over more than a century. Seven regional taxa

of the species group were dedicated to Tillyard by some of his colleagues over 90 years, from Martin (1906) to Theischinger (1995).

5.5. Etymology used by Tillyard

In the era of Tillyard's work, and earlier, it was rarely the practice to give the etymological derivation of the genus-group or species-group name. An exception which he sometimes used was to acknowledge a person after whom a species was named. Meanings of many of the names he proposed have to be deduced from likely Latin or Greek roots. Watson (1969) lists the location of primary type material, and designates lectotypes where necessary, for all Australasian dragonflies described by Tillyard. His paper serves as an excellent checklist to the dragonflies which Tillyard named. Tillyard (1916b) defines: "The prefixes *Noto*- [Gr. Νότος = south wind] and *Austro*- [L. australis = southern] may conveniently be used to denote purely Australian genera." Accordingly, he named nine genera with prefix *Austro*- and two with *Noto*- 44% of his 25 genus names. A thorough treatment of the etymology of the dragonflies named by Tillyard is given by Endersby (2012).

If his 129 species-group names are categorized, eponyms (named after people) and colourings each number 25. Morphology is next with 24 and four others use the colour of a morphological character. Size or appearance account for another 8. There are 13 species which are toponyms (named for a place) in the broad sense with another 5 relating to a direction: eastern, western. Unlike his European predecessors Tillyard had the privilege of seeing his specimens alive allowing him to name some for their behaviour (3) or their habitat or substrate on which they were commonly found (10). Describing colour, which can fade substantially in some museum specimens, also was made more definitive by reference to live specimens. That in total accounts for all but 12 of the specimens he named. [*Austrophya*] *mystica* still remains a puzzle, perhaps referring to the obliterated markings on the sole, aged and damaged female specimen. The remaining eleven species names recognize a relationship with other species.

Tillyard recognized the concepts of evolution and phylogeny, but perhaps not in a modern sense, even though he used the terms in some of his original descriptions. He defined "*Asthenogenesis* as the development of a successful line of descent by the adoption of weakness in structure." The term is not in current use, nor is it readily understood. Another early author interpreted it as meaning a reference to convergence through the loss of characters or neoteny. Tillyard writes of "a specialized asthenogenetic offshoot from the main stock", "one of the first

asthenogenetic offshoots from that type" and "a form *asthenogenetically intermediate* between the less-reduced *Lestes* and the more reduced *Protoneura*". In another description he suggests "a more archaic form … has either evolved gradually from ..; a more useful form, or has arisen as a sudden mutation, or, possibly, as the direct inheritance of an acquired character, in the Lamarckian sense".

5.6. History of odonatology

5.6.1. Updated history of odonatology

A detailed history of odonatology, modestly called 'A short history of odonatology', was published by Corbet (1991) in which he identifies six strands describing the progress of the field. Below, we summarize each strand and provide some updates corresponding to important events that took place after the publication of Corbet (1991).

The first one was named the *exploring strand* and it refers to the period when attributes of odonates such as shape, mode of reproduction and development were recognized. During this period, there was no rational system for naming species. The span of this strand was not defined. The *codifying strand* starts in 1758 with the introduction of the binomial nomenclature by Linnaeus (1758) who described only 18 species that he placed in the genus *Libellula*. The number of described odonates increased during this period, especially with the descriptions of J. C. Fabricius who, by 1798, had a list of 69 species (Kirby 1901). Fabricius was the first to give to dragonflies a separate ordinal status with the name 'Odonata' in 1793. The *classifying strand* starts in 1820 and was founded by Baron Michel Edmond de Selys-Longchamps who realized that wing venation is a reliable trait for classification of several families of odonates. During his life, Selys described 700 species and established 134 valid genera (Wasscher and Dumont 2013). For more than a century and a half, wing venation was used as the key trait to classify families and genera. In fact, there have been many attempts to establish the phylogeny of odonates using morphological traits (Fraser 1957, Hennig 1969, Hennig et al. 1981, Pfau 1991, Trueman 1996). However, recent phylogenetic studies have shown morphology alone, even with extensive analyses (Rehn 2003), does not reflect the true phylogeny (Fleck 2004, Dijkstra and Vick 2006, Pilgrim and Von Dohlen 2007, Ware et al. 2007, Carle et al. 2008, Fleck et al. 2008) , and can totally overlook cryptic species (Yong et al. 2014). For instance, wing venation is not a highly reliable trait because it evolved multiple times (Ware et al. 2007). After a long history of systematics, the classification of odonates has improved substantially by extensive phylogenetic studies (Ware et al. 2007, Fleck et al. 2008, Dijkstra et al. 2014). A decade ago, Trueman (2007) predicted that all major issues related

to odonate classification worldwide will be resolved by 2017 and at most 2027. Although a lot has been done during the last decade, we probably need another decade to provide the definitive global phylogenetics of the order.

In the *integrating strand* (1913), odonatologists started to compile observations on ecology, behaviour and physiology of species. R. J Tillyard, named 'a giant' by Corbet (2003b) basically established this strand with his classic book '*The Biology of Dragonflies*' (Tillyard 1917a). Corbet is another important figure of this period who published a book (Corbet 1962) and a review (Corbet 1980) that encompass the biology, ecology and behaviour of odonates. In addition, two main books have been published during the past two decades (Corbet 1999, Córdoba-Aguilar 2008). First, 'Dragonflies: Behaviour and Ecology of Odonata' (Corbet 1999) compiles information of all fields and treats all aspects of odonatology, except for morphology and physiology which have only been briefly discussed. It is probably the most used book by contemporary odonatologists. The second book is 'Dragonflies and damselflies. Model organisms for ecological and evolutionary research' edited by Alex Cordoba-Aguilar and published in 2008, the same year in which Corbet passed away. The book contains 20 chapters written by many experts in Odonata research, encompassing reviews of modern studies in systematics, evolutionary biology, ecology, conservation biology, and behaviour.

The *intercommunicating strand* which may be said to mainly start with the foundation of the Societas Internationalis Odonatologica (S.I.O.) in 1971 (Kiauta and Van Brink 1972) which launched the journal *Odonatologica* in 1972 whose aim is to promote and facilitate the international exchange of knowledge about odonates. In 1998, another major peer-reviewed journal named *International Journal of Odonatology* was founded. This quarterly journal promotes the publication of papers that address various subjects related to the study of odonates. In addition, regular national and international meetings are organized with the specific goal to provide a scientific forum and promote interactive exchange of ideas and results between scientists and amateurs. The International Congress of Odonatology is the largest meeting where odonatologists of different nations assemble to present projects related to the exploration and protection of the dragonflies of the world and their habitats.

The final *conserving strand* started with the formation of the Odonata specialist group within the IUCN in 1980 to determine conservation priorities for dragonflies and damselflies that ameliorate habitat degradation resulting from human activities (Moore 1982). Odonates have

played an important role in systematic conservation planning (Prendergast et al. 1993), and their protection benefited many other invertebrates and vertebrates of freshwater ecosystem (Knight et al. 2005, Taylor 2006). It has been shown that dragonflies and damselflies are sensitive indicators of landscape change (Samways et al. 2005). Nowadays, the conservation of dragonflies and their habitats (Moore 1997) is established in many areas. Furthermore, the rapid increase of people awareness about dragonflies and their conservation during the last decade has been a major event that marked the contemporary development of this strand. Many scientific and non-scientific volunteers participate in data collection worldwide and contribute to the elaboration of databases of crucial scientific importance to solve questions related to timely environmental issues and improve human well-being. Citizen science seems to go hand in hand with odonatology and is likely the future for improving our knowledge about dragonflies and damselflies of areas that have never been explored, synchronizing sampling efforts at different parts of the world, and providing services that regular funding could not cover.

5.6.2. A handy history of Odonatology

The history of odonatology can be summarized in four eras: Selys era (systematics), Tillyard era (biology), Corbet era (behaviour and ecology), and blossoming era (volunteering, phylogenetics and climate change) (Fig. 3). Each era starts when its main contribution to odonatology was published, and stops when the subsequent significant contribution came out. However, the influence of each era never stops since the advances of each era have built on the basic knowledge for subsequent eras and formed the current science of odonatology. The three first eras were named after the name of scientists, but the fourth was not. It was called the blossoming era because it is characterized not only by an extraordinary increase in the popularity of odonates in scientific studies, but also by the overwhelming contributions of volunteers and citizen scientists coming from different backgrounds. We have included the historical timeline of peer-reviewed journals that are still active today as well as regular scientific congresses. Finally, our representation is just a simplification and neither includes the hundreds of field guides, atlases and Red Lists that are published frequently worldwide, nor the hundreds of names of odonatologists who have made outstanding contributions to odonatology in particular and science in general.

5.7. Prominent advances in odonatology

During the past 50 years, there have been major advances in the knowledge of odonates in systematics but also in various biological, ecological and behavioural aspects. We present seven most significant findings that we believe Tillyard would love to see if he were still alive.

Phylogenetics has played a crucial role in the understanding of the evolutionary relationships among odonate species (Kambhampati and Charlton 1999, Artiss et al. 2001, Ware et al. 2007, Fleck et al. 2008, Dijkstra et al. 2014). The use of sequences of base pairs besides morphological traits like wing veins, aedeagus, and colors to set a clear-cut phylogeny was certainly something that Tillyard would be delighted to use.

Odonate diversity on earth is estimated to be 7500 species (Clausnitzer et al. 2009). Recently, 60 new species were discovered in Africa, which represents the largest number of species to be named at once in 130 years (Dijkstra et al. 2015). Surprisingly, these species are far from being cryptic and can easily be identifiable with handy characters. This amazing discovery which motivated odonatologists worldwide indicates that more species are to be discovered, especially in areas where the risk of extinction is high.

Sperm displacement was first discovered by Waage (1979) in *Calopteryx maculata*. He noticed that the aedeagus of the damselfly possesses spines that remove sperm from female spermathecae with an efficiency of 99%. So, a male removes sperm of previous mates before he transfers his own sperm. Since its discovery, sperm competition has been recorded in many other odonates (Miller and Miller 1981, Miller 1987, Cordero et al. 1995) and theories of co-evolution between the sexes were advanced, suggesting that the end result of such an elaborative shape of male penis is the ability of the females to store sperm from previous matings (Córdoba-Aguilar et al. 2003). The finding of Waage (1979) opened a new field of research on reproductive behaviour and sexual selection that has been explored thoroughly afterwards in other taxa (Simmons 2001).

Seasonal regulation is a topic that has received much attention during the past few decades. Odonates are of tropical origin and have successfully colonized temperate regions and adapted to seasonality. Seasonal regulation means that species and/or populations adjust their life history according to local environmental conditions (Pritchard 1982, Norling 1984), often imposed by latitude and elevation. Three types of odonate life cycle have been distinguished (Corbet 2003a). Spring species are those that spend the last winter before emergence as a final stadium (F-0) and consequently typically emerge synchronously and early. Summer species, on the other hand, spend

the last winter in the stadium before F-0 and consequently emerge later asynchronously. Obligatory univoltine species have one year life cycle wherever they occur along the latitudinal or elevational gradient.

Thermoregulation strategies in odonates are among the most amazing in insects. Odonates thermoregulate to maintain their body temperature within the range that maximizes their activity and fitness. For instance, in Libellulidae the posture of the body is dependent on the position of the sun so that individuals of some species may minimize or maximize their exposure according to their thermal needs (May 1976). Thermoregulation is mainly determined by climate, body size and behaviour (Corbet 1999). This aspect of odonatology has benefited mostly from the classic studies of O'Farrell A. F. (1964), Veron J. E. N. (1973, 1974), and May M. L. (May 1976, 1977, 1979).

Conservation biology of odonates has been recognized for a long time as a priority (Moore 1982) for many reasons such as their cosmopolitan distribution, their key trophic position within the food web (as predators and prey) and their excellent reliability in indicating water quality (Samways 2008). Odonates have played an important role in systematic conservation planning (Prendergast et al. 1993). The protection of dragonflies and damselflies benefits many other invertebrates and vertebrates of freshwater ecosystems.

The blossoming era is also a period that Tillyard would be amazed to witness. The increasing interest in dragonflies by scientists and amateurs has reached a point that has never been attained in the history of odonatology.

5.8. The influence of the book on non-scientists

Here we report an anecdote which shows the importance of the book to the public. In the preface of the book, Tillyard wrote "*The MS. of this book was completed on March 19th, 1915*…". In March 2015, exactly 100 years to the month later, an amazing video that beautifully illustrates and animates several figures of the '*The Biology of Dragonflies*' was posted (access this video here: http://juliagoschke.com/2015/03/the-biology-of-dragonflies/). The artist, Julia Goschke, was contacted to enquire whether she knew about the author, the book, or dragonflies. She answered: '*I'm just an illustrator and animator and I was inspired by the nice illustrations of this book. I just had the idea of making a piece of a dragonfly, because I like these animals*' (pers. comm.). As a matter of fact, it is easily detectable from her video that Ms. Goschke was not an entomologist since she included two figures of another insect taxon (Neuroptera) which do not belong to the

book. Ms. Goschke was not aware of Tillyard's biography or when he completed his book. It was just a spontaneous inspiration that created an exceptional coincidence, showing that the book has not only inspired many scientists over decades, but is still influencing nonscientists even today.

5.9. Future directions

After 260 years of taxonomy of odonates, we are on the verge of completing the global phylogeny of one of the most ancient of winged insect groups (Grimaldi and Engel 2005). Some taxonomic issues and uncertainties have yet to be resolved and a consensus for the reclassification must be attained before we witness this historical event. Since molecular results showed that wing venation is not a reliable criterion for classification, the next challenge is also to revise the taxonomy of fossil Odonata.

The priority for future research topics should include the understanding of geographic patterns of the dynamics of phenology and range distribution of odonates and the implication of climate change. Environmental factors that govern the direction and intensity of phenological response have to be investigated in detail to predict large scale changes in phenology and their consequences on the food web. Since odonates are predators and prey in both terrestrial and aquatic habitats, their phenological shift may lead to mistiming between trophic levels in terrestrial and aquatic system and local species extinction. Furthermore, the rapid range shift of different species of odonates to the north and higher elevations is also alarming because of the lack of information about the potential community interactions between invaders and residents. Considerable efforts should be devoted to the taxonomy of tropical species, which have received much less attention than temperate species. Because tropical species are thought to be more vulnerable to warming than temperate species (Deutsch et al. 2008), many may go extinct before being discovered. Finally, habitat fragmentation and water pollution represent huge extinction forces that threaten species persistence in various freshwater systems. Conservationists should develop a new practical theory that encompasses problem-orientated approaches rather than a fixed protocol for all species, taking into account the habitat, community (not a single species), sensitization, and cultural background of local people.

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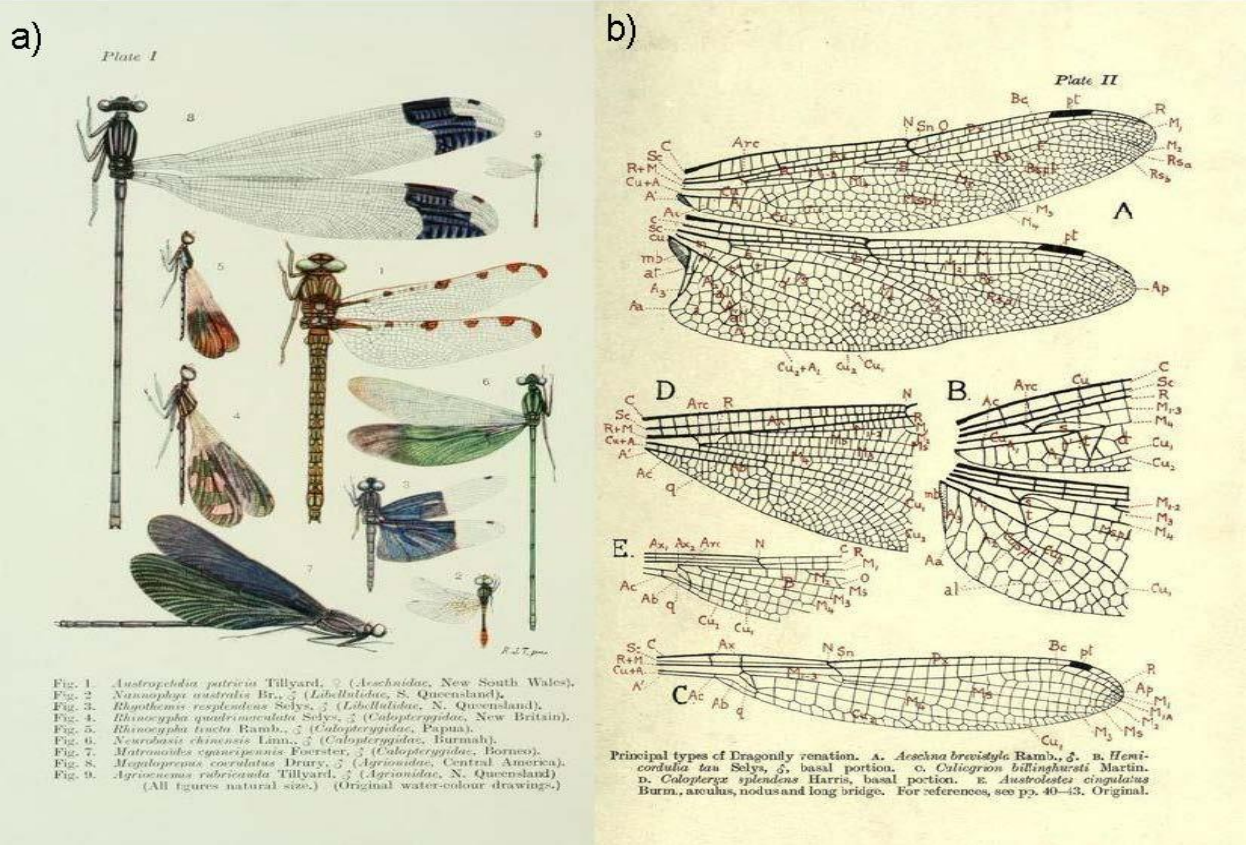

Figure 1. (a) Plate 1 (frontispiece) of the Book. Nine species are illustrated: *1. Austropetalia patricia* Tillyard, 1910; 2. *Nannophya australis* Brauer, 1865; 3. *Rhyothemis resplendens* Selys, 1878, 4. *Rhinocypha quadrimaculata* Selys, 1853; 5. *Rhinocypha tincta* Rambur, 1842; 6. *Neurobasis chinensis* Linnaeus, 1758; 7. *Matronoides cyaneipennis* Foerster, 1897; 8. *Megaloprepus caerulatus* Drury, 1782; 9. *Agriocnemis rubricauda* Tillyard, 1913. (b) Plate 2 of the Book. The terminology of wing venation is given in detail for A. *Aeshna brevistyla* Ramb. male; B. *Hemicordulia tau* Selys male, basal portion; C. *Caliagrion billinghursti* Martin. D. *Calopteryx splendens* Harris, basal portion. E. *Austrolestes cingulatus* Burm., arculus, nodus and long bridge.

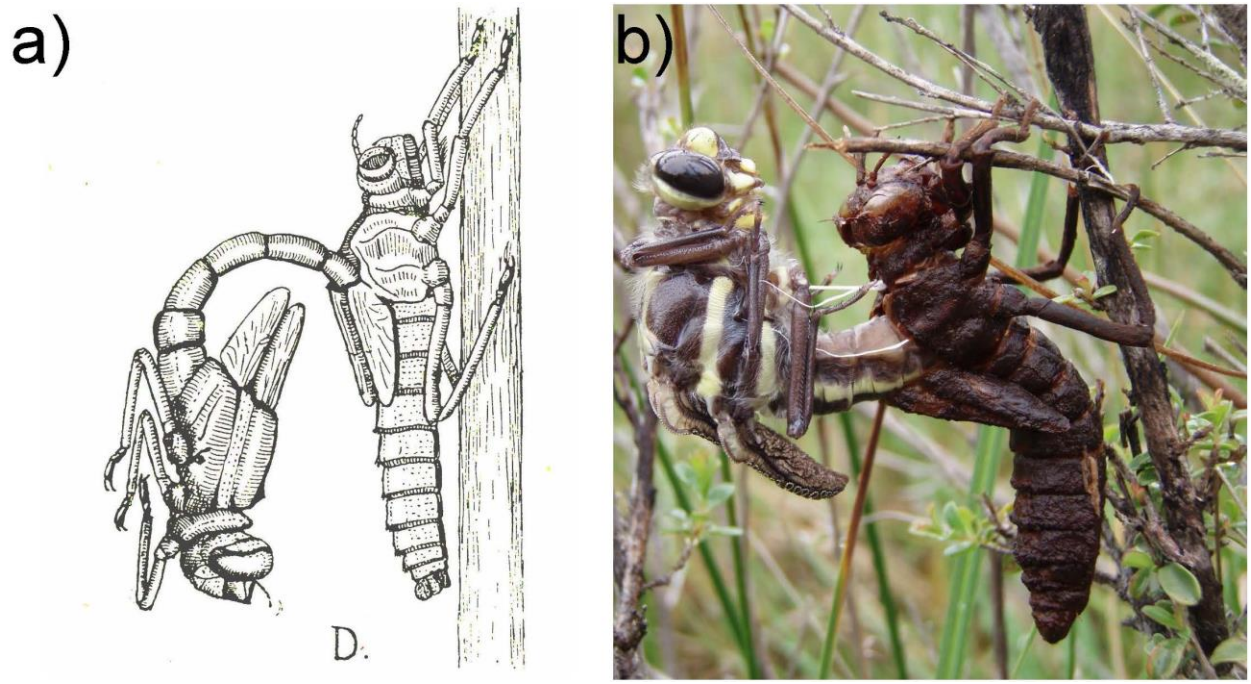

Figure 2. Metamorphosis of upright *Petalura gigantea*. (a) Hanging back position emergence as illustrated by Tillyard (1917a), page 94, figure 43D; (b) upright emergence as described by Baird (2013).

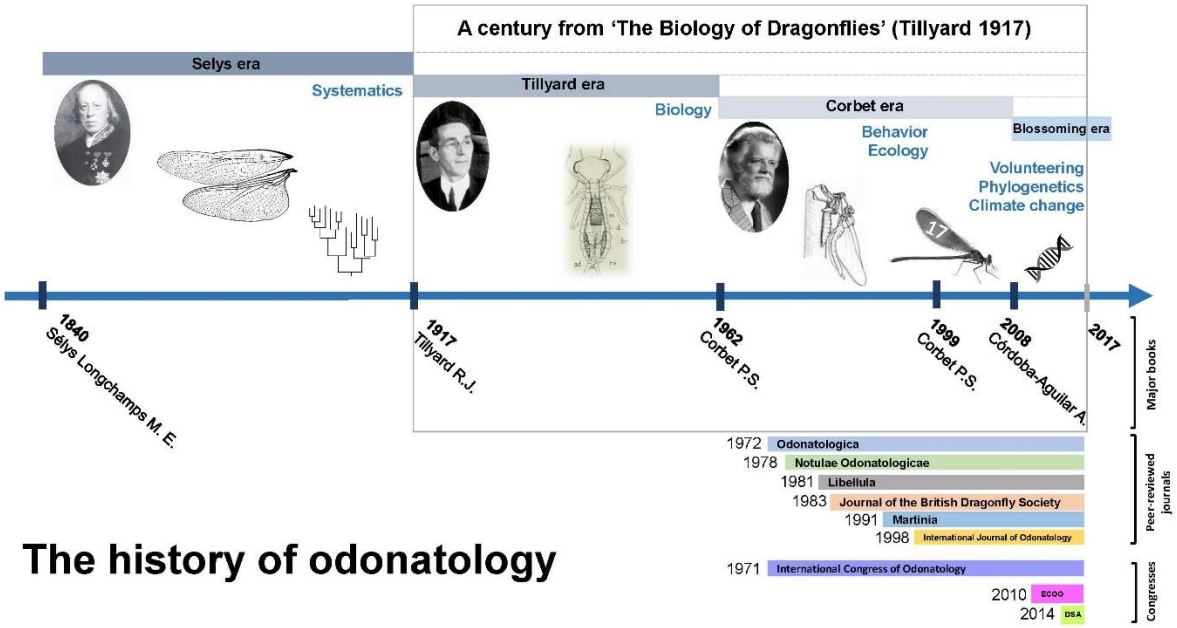

Figure 3. A history of odonatology. Four main eras are defined: Selys era, Tillyard era, Corbet era, and blossoming era. Although eras were limited in time in this representation, the dashed lines indicate that the influence of each era does not stop. Five major books are cited (Selys Longchamps 1840; Tillyard 1917; Corbet 1962, 1999; Cordoba-Aguilar 2008). Only peer-reviewed journals that are still active today are shown, among which *Odonatologica* and *International Journal of Odonatology* are the most popular. Three international congresses are noted. ECOO: European congress on odonatology, DSA: Dragonfly Society of America.

Table 1. Chapters of the Biology of Dragonflies (Tillyard 1917).

Number	**Title**	Page
1	Introduction	1
2	The Imago - External Features and Skeleton	9
3	The Wings	38
4	The Larva or Nymph	67
5	The Alimentary and Excretory Systems	100
6	The Nervous System	120
7	The Sense Organs	137
8	The Circulatory System	157
9	The Respiratory System	166
10	The Body-Wall and Muscles	202
11	The Reproductive System	212
12	Embryology	229
13	Coloration	243
14	Classification	258
15	Zoogeographical Distribution	281
16	The Geological Record	301
17	Bionomics, ETC.	321
18	British Species	338
19	Collecting, Rearing and Biological Methods	353

Table 2. Australian larvae described by Tillyard. The reference Tillyard 1917a in bold refers to his book 'The Biology of Dragonflies'.

Family	Species	References
Lestidae	*Austrolestes analis* (Rambur, 1842)	(Tillyard 1906, **1917a**, 1932)
	Austrolestes cingulatus (Burmeister, 1839)	(Tillyard 1906, 1914, **1917a**, 1917b, 1926)
	Austrolestes leda (Selys, 1862)	(Tillyard 1906, **1917a**)
	Austrolestes psyche (Hagen, 1862)	(Tillyard **1917a**, 1917b)
Hemiphlebiidae	*Hemiphlebia mirabilis* (Selys, 1869)	(Tillyard 1928)
Synlestidae	*Synlestes weyersii* (Selys, 1869)	(Tillyard 1914, **1917a**, 1917b, 1926)
Argiolestidae	*Austroargiolestes icteromelas* (Selys, 1862)	(Tillyard **1917a**, 1917b, 1926, 1932), as *Argiolestes icteromelas*
	Griseargiolestes griseus (Hagen, 1862)	(Tillyard 1914, **1917a**), as *Argiolestes griseus*
Lestoideidae	*Adiphlebia lestoides* (Selys, 1853)	(Tillyard 1909, 1912, 1915a, **1917a**, 1926)
	Diphlebia nymphoides (Tillyard, 1912)	(Tillyard 1912)
Isostictidae	*Neosticta canescens* (Tillyard, 1913)	(Tillyard 1914, **1917a**, 1917b)
Coenagrionidae	*Argiocnemis rubescens* (Selys, 1877)	(Tillyard **1917a**, 1917b)
	Austroagrion watsoni (Lieftinck, 1982)	(Tillyard 1917b), as *A. cyane*
	Austrocnemis splendida (Martin, 1901)	(Tillyard 1917b)
	Caliagrion billinghursti (Martin, 1901)	(Tillyard 1914, **1917a**, 1917b, 1926)
	Ischnura aurora (Brauer, 1865)	(Tillyard **1917a**)
	Ischnura heterosticta (Burmeister, 1839)	(Tillyard **1917a**, 1917b)
Austropetaliidae	*Austropetalia patricia* (Tillyard, 1910)	(Tillyard 1910c, **1917a**, 1917b, 1926)
Aeshnidae	*Anax papuensis* (Burmeister, 1839)	(Tillyard 1916b, a, 1917a, Tillyard 1932) as *Hemianax papuensis*
	Gynacantha dobsoni (Fraser, 1951)	(Tillyard 1916a, 1917a), as *G. rosenbergi*
	Dendroaeschna conspersa (Tillyard, 1907)	(Tillyard 1914, 1916b, a, 1917a)
	Austroaeschna obscura (Theischinger, 1982)	(Tillyard 1916b, a, 1917a) as *A. multipunctata*
	Austroaeschna parvistigma (Selys, 1883)	(Tillyard 1916a)
	Austroaeschna subapicalis (Theischinger, 1982)	(Tillyard 1916a), as *A. atrata*
	Austroaeschna unicornis (Martin, 1901)	(Tillyard 1916a), as *A. longissima*
	Austroaeschna pulchra (Tillyard, 1909)	(Tillyard 1916a), as *A. unicornis*
	Austrophlebia costalis (Tillyard, 1907)	(Tillyard 1916a)
	Notoaeschna geminata (Theischinger, 1982)	(Tillyard 1916a), as *N. sagittata*
	Telephlebia godeffroyi (Selys, 1883)	(Tillyard 1916a)

Gomphidae	*Ictinogomphus australis* (Selys, 1873)	(Tillyard 1917a), as *Ictinus australis*
	Antipodogomphus acolythus (Martin, 1901)	(Tillyard 1917a), as *Austrogomphus manifestus*
	Austrogomphus ochraceus (Selys, 1869)	(Tillyard 1916a, 1917a, 1926)
	Hemigomphus heteroclytus (Selys, 1854)	(Tillyard 1910c, 1914, 1916b, 1917a)
Petaluridae	*Petalura gigantea* (Leach, 1815)	(Tillyard 1909a, 1910c, 1911 1917a)
Synthemistidae	*Archaeosynthemis orientalis* (Tillyard, 1910)	(Tillyard 1910a, 1914, 1916b, 1917a), as *Synthemis macrostigma*
	Choristhemis flavoterminata (Martin, 1901)	(Tillyard 1910a)
	Eusynthemis tillyardi (Theischinger, 1995)	(Tillyard 1910c, 1916b), as *E. guttata*
	Parasynthemis regina (Selys, 1874)	(Tillyard 1910c), as *Synthemis regina*
	Synthemis eustalacta (Burmeister, 1839)	(Tillyard 1910c, 1917a, 1926)
Corduliidae	*Hemicordulia tau* (Selys, 1871)	(Tillyard 1914, 1915b, 1916b, 1917a, 1926, Tillyard 1932)
Libellulidae	*Diplacodes bipunctata* (Brauer, 1865)	(Tillyard 1917a, 1926)
	Diplacodes haematodes (Burmeister, 1839)	(Tillyard 1914, 1916b, 1917a)
	Nannophlebia risi (Tillyard, 1913)	(Tillyard 1913)
	Orthetrum caledonicum (Brauer, 1865)	(Tillyard 1916b, 1917a)
	Tramea loewii (Kaup, 1866)	(Tillyard 1917a, 1926)
Genera incertae sedis	*Cordulephya montana* (Tillyard, 1911)	(Tillyard 1911, 1917a)
	Cordulephya pygmaea (Selys, 1870)	(Tillyard 1911, 1914, 1916b, 1917a)
	Austrocordulia refracta (Tillyard, 1909)	(Tillyard 1910b, 1914, 1916b, 1917a)

As much as Tillyard's largely adult based taxonomic studies of Australian dragonflies inspired, influenced and facilitated the work of subsequent generations of students of Australian dragonflies, his work on the larvae is the basis for all subsequent larval studies in Australia and the reason why larval taxonomy of Australian dragonflies is in reasonable shape and now useful for diversity, environment, conservation and even climate change studies (e.g. Bush *et al.* 2012).

Conclusions & Future Directions

The research presented in the current thesis shows a comparative analysis of phenological shift, thermal sensitivity of phenology, and thermal adaption of insects in latitude and elevation, and reveals the underlying relevant environmental factors that govern the temporal and geographic patterns.

Chapter I investigated the spatiotemporal pattern of odonate adult phenology in northcentral Europe using long-term observation data of 54 species and showed interesting opposing patterns of the magnitude of phenological shift with an increase across latitude and a decrease across elevation. Based on large-scale temperature data and information on the thermal performance curve of species, it was revealed that the spatiotemporal pattern of phenology was best explained by the changes in both the average and fluctuation of temperature. The importance of fluctuation of temperature in shaping life history of ectotherms has shown an increasing interest in recent years (Bozinovic et al. 2011, Thompson et al. 2013, Vasseur et al. 2014), and depending on the geographic location and local temperatures, it may either increase or decrease the response of species to warming. Given that ectotherms show similar shape of thermal performance curve, it is likely that the magnitude of phenological shift of a large number of ectotherms increases across latitude and decreases in elevation.

Chapter II showed that the sensitivity of phenology of odonates to temperature changed over time, revealing a decrease across latitude and no change across elevation. Three hypothesis were tested to understand the geographic pattern of physiological change in the response of species to temperature namely (1) environmental temperature dictates the response of species to warming; (2) photoperiod change due to phenological shift lead to an interaction between photoperiodic and thermal cues; (3) changes in winter temperature (chilling effect) affects the response of species to spring temperature. The analysis showed that hypothesis 3 and probably 2 play an important role in shaping thermal sensitivity of species to warming. A similar account was shown in plants (Fu et al. 2015), which suggests that changes in physiological response of organisms to warming might be general across animals and plants. Thus, future attempts to predict changes in phenology of organisms should take into account the potential interaction of spring temperature with environmental factors such as photoperiod and carry-over effects of cold temperatures.

Chapter III which was conducted on five species of *Sepsis* flies is a follow up to the previous two chapters and shows experimentally the effects of fluctuation of temperature and cold temperatures on thermal responses. Based on estimated thermal performance curves (TPC), it was shown that large fluctuations of temperatures increases the responses of species to temperature and leads to earlier emergence of adults. Then, the usefulness of TPC in predicting the effects of fluctuation of temperature was confirmed by comparing observed estimates in the laboratory and predicted estimates from TPC. Furthermore, using a field experiment on five species we tested the accuracy of TPC-based estimates compared to field observed estimates of development time, and we showed that the more temperature fluctuates towards colder temperatures (below critical minimum) the less accurate the TPC-based estimate become. In fact, cold temperature tended to make Sepsis flies emerge earlier than expected, which is in line with our findings with dragonflies in chapter two. In other insects, the relationship between cold temperatures and physiological response to temperature has also been highlighted (Bosch and Kemp 2003, Stålhandske et al. 2014), suggesting that the pattern might be widespread across insects and even ectotherms.

Chapter VI documented a new behavior in odonates which is sexual death feigning. Although death feigning is common among animals, its occurrence in sexual context, that is, one sex pretends to be dead to avoid the other sex, seems particularly rare. This behavior was discovered in the Swiss Alps in the Moorland hawker (*Aeshna juncea*), a common species in Europe. The evolution of such behavior is suggested to result from sexual conflict. Repeated copulation is known to be disadvantageous for females because it may lead to damages of reproductive tracts (Dagmar von and von Helversen 1991, Crudgington and Siva-Jothy 2000). Also, there are three main aspects of the reproductive behavior of the species that make females more prone to repeated matings: (1) males constantly harasses females, (2) males are more numerous than females in reproductive sites, and (3) females are not protected by their partner during oviposition. The potential survival costs and constant exposure to harassment may have led the female to evolve sexual death feigning. Since this behavior was discovered in a common species that has been investigated for a long time, it is likely that sexual death feigning is more common than the literature suggests and more behavioral surveys would reveal new cases in insects in particular and arthropods in general.

Chapter V highlights the importance of a pioneer book in odonatology – The Biology of Dragonflies by Tillyard (1917) – a hundered years after its publication. This remarkable

contribution set the scientific foundation for the biology and systematics of odonates and increased the interest of scientists and amateurs who have thoroughly studied this group during the last century. Nowadays, odonates are among the most investigated insects in evolutionnary and ecological studies. I now acknowledge Tillyard not only for his outstanding scientific endeavor but also for his devotion to science in spite of the several health issues that he had had during his life.

6.1. Future directions

The present dissertation has advanced ideas that shed light on relevant and timely environmental issues, but also has created new avenues for further investigations. If phenological shift is linked to fitness such that earlier emerging individuals survive better and produce more offspring, the opposing phenological shift of odonates in latitude and elevation suggests that the timing of species and populations living at the extremes of the two gradients will evolve differently (Réale et al. 2003, Franks et al. 2007). A common garden experiment on populations living at high latitude and elevation can reveal potential difference in development time. More challenging research would be to put the phenology of odonates in a context of ecological network and determine the potential effects of phenological shift on the interspecific relationship (Encinas-Viso et al. 2012, Rafferty and Ives 2013). The diversity, large distribution, abundance and the complex life cycle of odonates make them key trophic levels for both terrestrial and aquatic ecosystems (Corbet 1999). It is likely that the phenological shift of odonates may disrupt different ecological interactions among organisms leading to potential mismatches among trophic levels in aquatic and/or terrestrial habitat. If ecological networks are affected by phenological shift of odonates, then there might be differences in the response of ecological communities at high latitude and elevation given the difference in phenological shift.

Further research should also be devoted to the understanding of the spectrum of environmental factors that may interact with temperature and change the expected timing of species (Thackeray et al. 2016). Experiments should investigate the interplay of photoperiod and winter conditions and on the response of species to temperature and how they affect development and phenology. These information are crucial for the better prediction of future warming effect on phenology of species.

The relationship between of the plasticity potential and population trends of species has been highlighted (Møller et al. 2008), such that populations that did not show phenological

response to climate change have declined. With the same long-term dataset of odonates that I worked on in this dissertation, this hypothesis can be addressed in both latitude and elevation, and if this relationship holds true, field and experimental studies should determine whether the reasons of the population decline are abiotic (failure to track the thermal niche), biotic (disruption of community interaction) or both.

The core of this dissertation was based on long-term observation data made by professionals and citizens. This shows how important citizen science data can be and why it is crucial to promote it in the future at an even larger scale (Silvertown 2009, Conrad and Hilchey 2011). The understanding of climate change effects on biodiversity and the elaboration of solutions for the current environmental issues cannot be done only by scientists and this is why the engagement of citizens is key.

6.2. References

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2004-2009	**Ingénieur in Ecology (head of the class honors)** Department of Biology, University of Guelma, Algeria Ingénieur thesis: Odonatological inventory of the Seybouse watershed
2009-2012	**Magister in Ecology (head of the class honors)** Department of Biology, University of Guelma, Algeria Magister Thesis: Cartography of the Glossy ibis (*Plegadis falcinellus*) dispersal
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PUBLICATIONS

2017	**Khelifa R.** Faking death to avoid male coercion: extreme sexual conflict resolution in a dragonfly. Ecology 98, 1724-1726. **Khelifa R.,** Zebsa R., Amari H., Mellal M. K., Bensouilah S., Laouar A., Mahdjoub H (2017). Unravelling the drastic range retraction of an emblematic songbird of North Africa: potential threats to Afro-Palearctic migratory birds. Scientific Reports 7: 1092. **Khelifa R.**, Theischinger G. and Endersby I. (2017). A century on from The Biology of Dragonflies by Tillyard 1917: what have we learned since then? Austral Entomology 56, 138-147.

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AWARDS & GRANTS

2017	**SNSF early mobility postdoc** in University of British Columbia Topic : *The effects of global warming and urbanization on the distribution of dragonflies and damselflies*
2016	**Forschungskredit grant** for PhD extension (1 year) o Awarded by the University of Zurich, Switzerland.
2016	**Best oral presentation** in the European Congress on Odonatology, Sweden
2016	**Congress Grant** from the European Congress on Odonatology, Sweden
2013	**Swiss Government Excellence Scholarships** for PhD students (3 years) o Awarded by ESKAS, Swiss Government
2012	**Head of the Magister class** at the biology department, University of Guelma, Algeria
2009	**Magister position award**
2009	**Certificate of honor** for raking first of the Biology Department o Awarded by University of Guelma, Algeria